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PHYSIOLOGY

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JACQUES LOEB
EDITOR

VOLUME I

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BERKELEY
THE UNIVERSITY PRESS
1902-1903

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PHYSIOLOGY

Vol. 1, No. 1, pp. 1–3 April 27, 1903

ON A METHOD BY WHICH THE EGGS OF A SEA-URCHIN *(STRONGYLOCENTROTUS PURPURATUS)* CAN BE FERTILIZED WITH THE SPERM OF A STARFISH *(ASTERIAS OCHRACEA)*.

BY

JACQUES LOEB.

It is well known that it has thus far been impossible to hybridize animals which are not in close blood relationship. Yet, it seemed to me that in order to obtain more definite data for the problem of evolution the apparent barriers to a successful hybridization of not closely related animals should be removed. For a number of years I have made experiments in this direction, and on one occasion—in my paper on The Artificial Parthenogenesis in *Annelids* 1901—I mentioned some of them.

Last summer it occurred to me that possibly the idea which guided me in my experiments on artificial parthenogenesis and other problems might also lead to positive results in this case, namely, that a slight change in the constitution of the sea-water (or the blood) may give the tissues properties which they do not possess ordinarily. This assumption was correct and I have recently succeeded in fertilizing the eggs of a sea-urchin (*Strongylocentrotus purpuratus*) with the sperm of a starfish (*Asterias ochracea*). The main results of my experiments may be summarized as follows:

1. It is impossible to fertilize the eggs of *Strongylocentrotus purpuratus* with the sperm of *Asterias* (or of any other of the starfish tried) in normal seawater.

2. I have found a series of solutions in which the eggs of *S. purpuratus* can be fertilized with the sperm of *Asteria ochracea*.

3. The seawater contains certain substances which if added to these solutions prevent the fertilization of the egg of *S. purpuratus* with the sperm of *Asterias ochracea*.

4. The same solutions in which the eggs of *S. purpurata* can be fertilized by the sperm of the starfish render difficult or impossible the fertilization of the same eggs by the sperm of their own species. But in the same solutions the eggs of *S. purpuratus* are very readily fertilized by the sperm of their own species, when those constituents of the seawater are added which inhibit the hybridization of the egg of the sea-urchin with the sperm of the starfish.

5. In some experiments as many as fifty per cent. of the eggs of the sea-urchin formed the characteristic membrane of fertilization in the artificial solutions in about half an hour after the sperm of the starfish had been added. The same eggs began to segment at the proper time while the eggs without a membrane remained unaltered. Under proper conditions practically each of these segmenting eggs reached the blastula stage and many the gastrula stage. Some remained alive for more than a week and showed the differentiation of the intestine. Inasmuch as the parthenogenetic larvae of *S. purpuratus*, as well as the pure-blooded larvae of this species, form a skeleton while the larvae of the starfish form no skeleton, it may be of interest that the hybrids between the two formed no, or at the best, only a very rudimentary skeleton. It is not impossible that the spermatozoön of the starfish contains substances which interfere with the formation of a skeleton.

6. Each series of experiments was accompanied by the necessary control experiments to exclude the two possible sources of error, namely, contamination of the eggs by sperm of their own species and second, artificial parthenogenesis. The first source of error was excluded by the fact that in the experiments in which only the sperm of *Asterias* was used, no egg formed a membrane

or developed in normal seawater. The second error was excluded by the fact that, when the eggs were kept in the artificial solutions without the addition of the sperm of *Asterias*, no egg formed a membrane or developed. As soon, however, as the sperm of *Asterias* was added membranes were formed and the eggs began to segment at the proper time.

These facts may also have a bearing upon certain phenomena of infection and immunity. One is almost tempted to say that while in seawater the eggs of *S. purpuratus* are immune against the sperm of the starfish, in the artificial solutions they become almost or completely immune against the sperm of their own species, and at the same time lose their immunity for the sperm of the starfish. It is a rather remarkable fact that surprisingly small though very definite quantities of certain constituents of the seawater suffice to reverse the character of the immunity.

As a brief statement of the chemical side of these experiments is almost impossible, I will reserve it for the fuller publication which is in preparation and will appear shortly.

The experiments are being continued.

University of California,

Berkeley, California, April 27, 1903.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 2, pp. 5–6 May 25, 1903

ON THE MECHANISM OF THE ACTION OF SALINE PURGATIVES, AND THE COUNTERACTION OF THEIR EFFECT BY CALCIUM.

BY

JOHN BRUCE MacCALLUM, M.D.

(From the Physiological Laboratory of the University of California.)

1. It is commonly believed that saline purgatives act locally, and directly upon the intestine. According to Schmiedeberg, Cushny and others, they have a purgative action because they prevent the absorption of fluids from the intestine. Loeb has stated that these purgatives are identical with the salts which produce contact irritability, muscular twitchings and hypersensitiveness of the nervous system; and he has suggested that they increase peristalsis by increasing the irritability of the nerves and muscles of the intestine.

2. I have made a series of experiments on rabbits to decide the question. They show that barium chloride, sodium citrate, fluoride, sulphate, tartrate, oxalate, and phosphate, act as purgatives not only when introduced into the intestine, but also when injected subcutaneously or intravenously.

3. The intensity of their action is greatest with barium chloride and decreases approximately in the order named.

4. The purgative action of these salts is caused first by an increase in peristalsis and second by increased secretion of fluid into the intestine, both of which can be directly observed.

5. Although I have not specially studied the secretion of other glands, I have noticed that in a number of cases an increased flow of saliva and urine occurred when these salts were introduced into the body.

6. Intravenous injection of 1–2^{cc} $\frac{M}{8}$ solution of these salts causes increased peristalis within one minute. When introduced into the intestine it takes 10–15 minutes and five times the amount to produce an equal effect.

7.—This seems to indicate that even when these salts are introduced into the intestine, they must be absorbed into the blood before they can produce their purgative effect; and that they affect the intestine through the central or the peripheral nervous system as Loeb has suggested. Their action is not due to the prevention of the absorption of fluids from the intestine.

8. By the injection of solutions of calcium chloride these peristaltic movements can be entirely inhibited.

9. There is a perfect analogy between these actions and the production and suppression of muscular twitchings and nervous hypersensitiveness.

10. The administration of calcium is therefore rational especially in those cases of diarrhœa in human beings which accompany hysteria or nervous excitability of any sort.

11. Small doses (5^{cc} $\frac{M}{8}$ $CaCl_2$ solution containing about 7 centigrams of $CaCl_2$) introduced into the stomach of a rabbit inhibit peristalsis in 10–15 minutes and have no injurious effect.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 3, pp. 7–9 August 3, 1903

ARTIFICIAL PARTHENOGENESIS IN MOLLUSCS.

BY

JACQUES LOEB.

After the discovery of the fact that the unfertilized eggs of a sea-urchin can be caused to develop by merely extracting water from them and without the addition of sperm, the question arose whether artificial parthenogenisis is confined to a few species or whether it is as general as the phenomenon of sexual reproduction itself. If the latter could be shown to be the case it would be of importance in two directions. It would suggest the possibility that the artificial physical or chemical means by which the unfertilized eggs can be caused to develop have something in common with the means by which the sperm causes the development of the eggs. Thus the experiments on artificial parthenogenesis might pave the way for the solution of the problem of fertilization. Second, if it be possible ever to gain any positive evidence for abiogenesis the experiments on artificial parthenogenesis may have some bearing on this problem.

It can, of course, not be expected that the eggs of each of the numerous forms of animals can be examined in regard to the possibility of artificial parthenogenesis. If it can only be shown that the eggs of each form on which the experiment has been tried can be caused to develop without sperm by physical or chemical means we may conclude with some probability that the eggs of the animals of the same group not yet tested would behave in the same way, although the technical difficulties may be greater in one form than in another.

The forms thus far experimented upon all belong to two groups, Echinoderms and Annelids. No species of these two groups whose eggs have been subjected to the test for artificial parthenogenesis have proved refractory. I had long intended to see whether the unfertilized eggs of Molluscs could not be caused by physico-chemical means to develop, but at Wood's Hole no forms with separate sexes were obtainable. Meanwhile Ariola reported that he had tried in vain at Naples to produce artificial parthenogenesis in a Mollusc, Dentalium entalis.* It must be said, however, that this author, through a blunder on his part, did not use the right solutions and hence his experiments could not be successful. Professor Kostanecki† recently published a short paper announcing the fact that he had succeeded in producing the first segmentations in a mollusc, Mactra, by the method employed by me on sea-urchins. The fact that he did not succeed in causing the eggs to reach the larval stage he ascribed to the possibility that he did not use the optimal concentration for this form.

I have recently had an opportunity to try some experiments on the Limpets of Pacific Grove, namely, *Lottia gigantea* (owl-limpet) and three other limpets, some or all of which are species of Acmaea. In each of these forms I succeeded in producing swimming larvae from the unfertilized eggs by submitting them for two hours to sea water the concentration of which had been increased by the addition of KCl or NaCl.‡ In Lottia I had to add from 15 to 20 cc. of a 2½ m solution of KCl to 100 cc. of sea water; in the case of one of the other limpets it was necessary to add only 10 cc. of the 2½ m KCl solution to 100 cc. sea water. The optimal concentration therefore differs somewhat for each species. The temperature during these experiments varied between 15° and 19° C.

In from two to three hours after the eggs were taken out of the hypertonic solutions and put back into normal sea water segmentation began, which was very regular at least in the two

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*Ariola. Mittheil. a. d. Zoologischen Station zu Neapel. Vol. XV. p. 408, 1902.

†Kostanecki, Bullet. Ac. Sci. Krakau, 1902.

‡KCl gave better results or at least more embryos than NaCl.

and four cell stages. For the eight cell stage I did not obtain any satisfactory observations. In from 20 to 24 hours swimming larvae were found. In most cases the latter died within 48 hours; in one species only (Acmaea?) did I find larvae which showed a tendency to live longer. Death was caused or at least accompanied by a tendency of the cells to fall apart and to disintegrate. In older cultures were found a comparatively large number of partial larvae.

What the cause of this disintegration was I am not yet able to state. It is probable that the eggs had not yet reached their full maturity at the time of my experimentation. I am inclined to conclude this from the fact that I did not succeed in fertilizing the eggs of Lottia (on which the majority of experiments were carried out) with sperm of their own (or any other) species. It is perhaps worthy of note that the eggs which could not be fertilized with sperm could be caused to develop by a physical agency such as the extraction of water from the egg. This refractory behavior of the eggs toward sperm was in one respect welcome in these experiments as it added to the feeling of security that the precautions* against the contamination of the eggs with sperm were adequate. In the control experiments no segmented egg or swimming larva was found.

Treatment of the eggs with acid led only to a begining of segmentation. Mechanical agitation, as far as tried, had no effect.

I can therefore state that on the basis of these experiments the Molluscs may now be added to the list of animals in which it is possible by physico-chemical means to cause the unfertilized eggs to develop into swimming larvae. The experiments will be continued.

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*These precautions were identical with those mentioned in former papers. Owing to the smooth surface of the shell and the foot of the animal it is easy to wash it thoroughly with fresh water before laying bare the ovary.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 4, pp. 11–31 October 5, 1903

THE RELATIONS OF BIOLOGY AND THE NEIGHBORING SCIENCES.*

BY

WILHELM OSTWALD.

The event which we celebrate to-day is like the launching of a beautiful ship. The keel has been laid with care; the hull has been made lean and powerful; the engines and propeller are of the newest and best construction. But above all we know that the captain is a man who can guide the ship on the high seas of science with a firm and courageous hand; for we are all aware of the wonderful journeys he has already made, and what fabulous treasures he has thereby gained. We may thus not only wish, but with all the certainty that is possible in human affairs, we may also predict for our splendid ship, a favorable and successful voyage. Only one question remains to be answered: What will the weather be?

Gentlemen, it is a hazardous undertaking to predict the weather of the future. Moreover, our ship is so constructed that it is capable of withstanding storms, and sailing against adverse winds. But since its builders have put into its construction love and hope, it would be a great pleasure to us if we could find adequate grounds for predicting good fortune for our ship in this respect also. Here the wish must not influence the judgment;

*Address delivered at the Dedication of the Spreckels Physiological Laboratory of the University of California, by Wilhelm Ostwald, Professor of Physical Chemistry at the University of Leipzig. Translated by John Bruce MacCallum.

we must test as justly as we can, the conditions of the scientific weather. Perhaps then we may learn in what direction it will be best for the ship to steer, that we may expect a successful journey.

A voyage of scientific discovery of to-day is like a journey taken five hundred years ago for the finding of new lands and seas. Unknown country is on all sides of us; and we shall see new things in whatever direction we pass beyond the boundaries of the known. For there is nothing easier than to make discoveries. He who cannot discover and classify new facts in any branch of natural science after a few weeks, or at most a few months, of industrious work must indeed be ignorant or unskilled. But more than this must be accomplished. New facts in themselves are only an insignificant part of a science. Their main value consists in their relation to what is already known, and to what will be known in the future. The value of new facts is like that of gold. Gold in itself is nothing more than one mineral among thousands; it is of value only in connection with the whole system of commercial values.

Thus if its only duty were to discover new lands, our ship might take any course it chose; it would be only a question of time when new land would be sighted. But it would be a matter also of chance as to what the nature of this land might prove to be. And a sceptical observer would perhaps say: One thing is certain, that we know nothing of this new land, and we should not attempt to make any predictions whatever concerning it.

To this we must answer that it is by no means so impossible to look into the future as we ordinarily think. If we go to the bottom of the matter we find that our whole civilization rests on the fact that we can and do look into the future. Indeed it may be said that the height of any civilization may be directly measured by the thoroughness with which the prophets of this civilization understand their calling, and are able to predict the future. With the exception of eating and drinking, sleeping and kissing, there are very few things that we do entirely for their own sakes. The countless other things with which we fill out our lives, are done by us only on account of the results we

expect from them in the future. This is what we have all done today when we prepared to come to this gathering. If you do not immediately arise and go home it is only because you expect to hear from myself and from others better things than you have heard before.

Naturally the degree of certainty with which one may prophesy is very different under different circumstances. That tomorrow and the next day, and every subsequent twenty-four hours, the sun will rise and set, that every 365 days the change of seasons will recur, we regard as so certain that we do not hesitate to arrange our entire lives on this basis. On the other hand we cannot, at least in Europe, plan a picnic two days in advance, because we do not know whether or not it will rain. That our countries will retain their financial credit for decades and probably for centuries, seems to us so certain that we do not hesitate to base our entire pecuniary existence on this assumption. What the exact condition of the stock markets will be, however, might perhaps be predicted a week ahead by a specially gifted broker; while the majority of men would be entirely unable to make any prediction.

Our ideas of the future consist of an enormously tangled and complicated tissue varying in its nature from the greatest certainty to the most doubtful possibility; and in every one of these various cases we must distinguish the *nature* and the *degree* of probability. We recognize at once that in the struggle for existence, that man will be most efficient who in any field can answer these questions: "What will happen?" and "With what degree of certainty will it happen?" more accurately than his fellow-men. Upon this rests the high veneration and the slavish fear in which races of low development hold their prophets and medicine men. When the Roman Republic undertook a war, it asked the oracles what the outcome of the war would be; and when in 1870 Bismarck saw that the conflict between Germany and France was unavoidable, he asked Moltke exactly the same question. The oracles predicted from the viscera of a sacrificial animal; Moltke made his prophecy on the ground of his scientific investigations of the military conditions of both countries. Here we have an illustration of the difference

between the old and the new civilization; and the word expressing this difference is *science*.

This is in fact the meaning of science; its aim is to make possible a glimpse into the future; and science is the more perfect, the more broadly and surely it allows us to obtain this view. The problem of seeking to find what is the essential thing in science has at some time concerned each one of us; and the answers to this question are very diverse. According to the condition of my knowledge I have found in the course of time that my answers have been very different. Since, however, I have possessed the definition which I have just given you, (which also I find given in Locke) I find myself in a position to solve a great number of different problems more easily and more surely than it was possible for me to do up to that time.

And now, gentlemen, though we have apparently wandered far from the subject of our discourse, we find ourselves again in the midst of it. Science must give us the means of choosing the course which our ship must hold in order to reach with the greatest certainty those unknown lands in which the greatest treasures lie; and when we have reached the sought-for coast, science must show us where we may best cast anchor, and how we may most easily make a landing.

Here we deal truly with a science of sciences. Special problems of mathematics or chemistry must not be solved here. On the contrary we must deal with problems concerning the laws according to which each individual science develops regardless of what it includes. Such a science hardly exists at the present time. There have appeared, it is true, valuable preliminary studies; the histories of the various sciences have been more or less thoroughly investigated, and *philosophy*, according to the modern definition, comes near to this problem. Still there is lacking for the most part the emphasizing of the history of development in this part of philosophy as well as the discussion of the points of similarity in the histories of the individual sciences. But from both sides there is undoubtedly present a tendency towards the advancement of work in this direction, and we may then look for the establishment of a *biology of the sciences* as a product of the near future.

It is in fact a *biological* problem with which we deal. A rock or a comet has no need of science, for as far as we are able to judge the existence of one of these bodies means nothing to the body itself. We on the other hand, who wish not only to maintain, but also to *improve* our conditions, need science for this purpose; for in order to retain anything, we must know the conditions under which it exists, and in order to improve it we must know how far it is possible to influence these conditions. The surest and broadest knowledge that it is possible for us to have of this is that which we call science.

In fact if we ask what is the most general force which has been active in historical times within our knowledge, and is still active, we recognise that it is the *conquest of all intellectual fields by science*. If we imagine the most primitive conditions in the development of mankind, we see that there is no doubt that *the* individual and *the* race which is finally successful in the struggle for existence is the one that learns to see most clearly into the conditions of the future and thus learns to influence them. There are conditions in which the war of physical force seems to settle the question; but even here we see *skill*, that is, the intellectual or scientific factor, offset a large part of the brute strength, and this factor increases as development advances. The greatest leaders of men have been those who saw most clearly into the future.

Perhaps the objection will be made here that we are accustomed to regard the law-makers and political organizers, and not those who make discoveries or inventions, as the leaders of men; and it is well to account for this apparent contradiction. We apply the same word *law* both to the regularities of the natural processes, and to the arbitrarily influenced actions of mankind. The great thing which the moral and political law-makers have done is *that they have made it possible in certain fields of human actions to make predictions*. Since they did not know the internal causes upon which such actions are dependent, they were obliged to make such external rules or laws in the *juristic* sense. These agree more or less with the unknown psychological and biological laws which are applicable to the phenomena in question. The better they have agreed,

the more lasting have been these laws, and the greater and more powerful have been the law-makers. Thus every political and moral organization is dependent on biological conditions; and these fields are evidently those which are destined to be irresistibly conquered by science.

Many of my friends, and indeed those whom I hold personally in very high esteem, have objected very strongly to these views when we have discussed them; and from this I presume that such an objection suggests itself to you also. We are in the habit of connecting with those questions which we have discussed, sensations which are of great value to us, and which we allow to exert a great influence upon us. We regard the political and moral laws with a feeling different from that with which we look upon physical and chemical laws. That which we call *reverence* distinguishes our attitude toward the former. And at the same time there is another great field which seems to be quite apart from the influence of science, and which, nevertheless, forms a great and precious part of our life. This is *art*. Here also more or less vivid sensations become active within us, and lead us to regard art as something which takes us beyond our usual selves, and our every-day conditions. These sensations of the beautiful, the great, the strong, the eternal, we do not wish to disturb or belittle; and therefore those who see the value and charm of life in those things, protest against their absorption by science, since the calmness and coldness which they ascribe to science are directly opposed to those sensations.

Now in a circle such as this where each one of us has in some way dedicated the better part of his life to science, I do not need to prove that science requires from her children soberness and coldness, so far as criticism is concerned, that is in testing whether work is trustworthy and sound. But no less does science demand of us *reverence*—reverence for the most lasting of all things we know, reverence for *truth*. This reverence arises in us from the knowledge that there is no escape from truth. It matters not how well hidden an error may be, or how perfectly disguised in the garb of truth, we all know that it will nevertheless be detected and banished; and that the inner life of science can drive forth with irresistible force such foreign

lodgers. And this expulsion indeed occurs the more quickly and more vigorously, the more intimate the communication is between the false and the true parts of the organism, because in this way the incompatibility sooner becomes evident. In the body of science only those errors which exist in an isolated structure connected with the other parts of science only mechanically, may remain there for a long time like an encap sulated foreign body. There is therefore no better means of testing the truth of a scientific observation than to place it in organic relation with as many other parts of science as possible. We shall have occasion to discuss this matter more thoroughly later.

Why this feeling of reverence does not arise in us with such strength when we think of science, as it does when we think of those things whose greatness and strength we have been accustomed to know from our youth up, is explained by the fact that we do not yet recognize the irresistible power of science as a matter of daily thought and experience. But when we have once learned to regard science as that which controls our whole existence, then we know that there is nothing more worthy of reverence, and nothing more powerful.

And now what is the position of the beautiful, of art? In the first place it is beyond all doubt that the development of art is dependent upon that of science. If one recalls the history of one of the completed epochs of art such as that of Greek sculpture, it is apparent that from step to step the growth of this art has depended upon increasing knowledge; upon the knowledge not only of the anatomy of the subject, the human body and its movements of expression, but also upon the knowledge of the materials, the marble and the metal. It is evident also that the degree of development of art corresponds with the scientific and technical development. Just as science in general requires for its advancement a constantly increasing knowledge of its subject and its materials, so it is with art. And just as in science a wonderful work may be done by a great genius with limited materials, such as the formulation of Newton's law of gravitation or Mayer's law of energy, so also a great artistic genius may with the limited materials of his time,

produce works which will live hundreds and perhaps thousands of years, works such as the dramas of Aeschylus or the Ninth Symphony of Beethoven.

Here too we are struck in a certain sense by the superiority of science; for while we cannot doubt that these products of scientific work will endure as long as civilization is present on the earth, we hesitate to make the same statement concerning the works of art. We cannot deny that in Aeschylus we are aware of a trace of antiquity. In other words there are passages which no longer affect us at the present day as they certainly moved the people of the time of Aeschylus. And in the same way it is not impossible to imagine that a time will come when the Ninth Symphony will affect men no more deeply than a symphony of Haydn now moves us.

This seems to indicate that perhaps art itself is destined to become slowly absorbed by science. I am inclined to believe that this idea will find vigorous and perhaps angry opposition. I emphasize the fact therefore that I personally am indebted to art for many uplifting and beautiful hours. Poetry, music and painting have given me refreshment and new courage, when exhausted by scientific work I have been obliged to lay my tools aside. But I cannot escape the conviction that this is a sign of imperfection, not so much an imperfection of science as of my own self. For science in the sense in which I speak of it today, exists only in its first beginning and the human organism has not advanced far in its adaptation to it. Thus each one of us suffers more or less from atavistic frailties, which find their clearest expression in our constant complaints concerning the immeasurable complexity and growth of scientific materials. We ourselves and our children, and our children's children shall still enjoy through art, hours that are beautiful and perhaps great; for there will always be fields of human experience into which science has not yet penetrated and in which art will have undisputed sway. But to a future race which has conquered the science of psychology, many of the valued psychological dramas of today will seem as naïve and unsatisfactory as the first attempts that a child of four years makes at drawing. But we must not dwell longer upon these

questions, because there are others more fundamental which are to be answered. We have discussed these matters only that they might be applied to the problem with which we are dealing, namely, the gaining of a clear idea as to the direction in which the science of *biology*, which we consider specially here, will probably advance. In order that we should have the right to make any statement concerning this, it has been necessary to show that such a prophecy is possible. And now one more small preliminary consideration brings us directly to the problem. Science is an organism which strives constantly for self preservation and development. It is therefore provided with organs of regulation, by which that which is useful is preserved and that which is harmful suppressed. It is possible for these organs to become active only when the processes themselves which are to be regulated come into activity. There is a certain delay in the working of each regulator. Thus it follows that science like every other self-regulating apparatus must necessarily undergo periodic variations from a middle point. This middle point is not necessarily unchangeable at all times. In our case it is undergoing a constant advance because science can only increase and not diminish in scope. Further there are here many sources of energy each with its own group of regulators; therefore we have to do with a number of super-imposed phases which form a very complicated picture. For example, by the summation of many maxima or minima which in themselves are small we obtain a very great elevation or depression. We shall try to determine what part of the curve of depression or elevation science in general, and biology in particular now occupy.

Now one of the very influential components of these movements is plainly to be recognized. We have just passed through a period in which all sciences have been isolated, a period of *specialisation*, and we find ourselves in an epoch in which the *synthetic* factors in science are gaining a constantly increasing significance. Consider, ladies and gentlemen, what you have before you. In the lecture room of a *biological* institute there speaks to you on this important occasion, a man who is not only not a biologist, but who on the contrary has been expressly

invited as the representative of a sister science. And further this man himself is the product of a synthesis of two neighboring sciences, namely physics and chemistry. Furthermore when this man seeks to bring to you the best which he can gather from his garden, he brings you no word of physics and chemistry, but rather thoughts which concern the triad, physics—chemistry —biology; and problems which include these and still more distant sciences. This is not an accidental thing, but a genuine expression of the efforts which fill all our days. Everywhere the individual sciences seek a point of contact with one another; everywhere the investigator determines the value which his special results may have in the solving of the most general problems. In short all sciences are tending to be philosophical. Nowhere is this tendency towards fundamental explanations so great as in biology. A glance at the literature of biology shows with what ardent care the biologists strive to throw light upon the fundamental problems of their work.

Thus the biologists consider it of the greatest importance to learn the relation which their science holds to the neighboring sciences, especially to the inorganic natural sciences, chemistry and physics; and the conflict of opinions has become marked by the watchwords, *vitalism* and *mechanism*. As is true in all such instances, one great danger arises, namely, that a complex of many things of most diverse character, is often named according to one of many properties by which it may be determined. In fact we see that a well-known investigator calls himself a mechanist while the mechanists think of him as a vitalist. When I seek to deal in my own way with the problems which lie before us, I prefer to remain neutral in relation to the two parties. Much more is it my object from the broadest standpoint to arrive at a true estimate of the relations of the sciences which we are considering.

In an attempt to make a general classification of the sciences, one soon arrives at the conclusion that they do not stand *side by side*, but that they *include* one another in a reciprocal manner which may be represented incompletely by geometrical figures. Human experiences are above all the objects of study for every science. Each of them is made up of an unlimited number of

component parts only a few of which according to the purpose of the science are taken into consideration. Thus the greatest number of experiences is included in that science which considers the smallest number of components. And it follows conversely that the smaller our range of choice in experiences is, the greater the number of components included by the sciences. Thus the science which from one point of view is the broadest is from the opposite standpoint the narrowest. The converse of this also is true.

In this wise you may note that on the one hand the theory of varieties (Mannigfaltigkeitslehre), by which I mean something of which mathematics or the theory of magnitudes is only a part, is the most comprehensive and the most narrow of all sciences, since it includes all experiences but considers them from only one standpoint (namely that each experience is an individual and separate thing). Psychology on the other hand is the narrowest and most comprehensive science because it considers only the phenomena which are connected with the existence of the human brain (or if one prefers it, with the human soul). Here however all possible components are taken into account. One may see at a glance that in the series of sciences with which the theory of varieties deals, each science pre-supposes the existence of that which precedes it in the series, but goes farther in that it comprehends new factors or new components of which the preceding science took no account. Its scope is therefore made narrower since it deals only with the subjects affected by these components. Mathematics thus has to do with varieties but only with those which possess magnitude. Geometry also deals with magnitudes, but only with those which have the properties of space. Physics treats of special objects only in so far as the various kinds of *energy* act upon them. Chemistry on the other hand deals with those physical objects which are distinguished from one another by qualitative differences with the exception of those differences due to the presence of different forms of energy. According to this system biology is that science which treats of those chemical objects which have a stationary condition of energy, that is, of nourishment and of reproduction. Finally, psychology deals with life processes only in so far as they affect

the functions of the mind. By this a practical and almost complete knowledge of the mental functions of man is arrived at, and this is almost the only thing of which we have certain knowledge.

It is scarcely necessary to say that this classification is arbitrary in that one may introduce numerous intermediate steps between those already given and in this way consider smaller differences. Thus one may interpose sociology and anthropology between biology and psychology, and physical chemistry between physics and chemistry, etc. I need not go farther into this matter. Of more importance perhaps would be the introduction between mathematics and geometry of chronological science, that is, the knowledge of the laws of *time* (which is not to be confounded with historical, geological or astronomical time). Still these considerations would carry us too far.

The most essential thing in connection with our problem, however, is that we should recognize plainly the general relations of biology. It includes all sciences from the theory of varieties to chemistry, that is, all biological phenomena are comprehended by the laws of these sciences. In exactly the same way all chemical phenomena are subject to the laws of mathematics and physics in so far as no chemical phenomenon will, if it is well founded, be contradicted by these laws. But the laws of mathematics and physics do not exhaust that which can be said concerning chemical phenomena; and the qualitative material differences which form the object of chemical study cannot be completely worked out by the means which these sciences afford. The cause of this lies in the fact that in chemistry we have a science of more manifold character than in the preceding sciences. It makes no difference whether a sphere of gold or one of carbon of equal size be charged with a hundred volts of electricity so long as we consider only the physical, that is, in this case, the electrical phenomena. But there is an irreconcilable difference, from the chemical point of view, between *burning* a sphere of sulphur and an equally large sphere of carbon. In the same way chemical composition does not distinguish a living man from a dead man, but biologically there is a fundamental difference. The former is

capable of nourishing and reproducing himself; the latter has not this power. You will perhaps here make some objections; but I do not believe that you will be able to make any essential change in this statement; at most it might be necessary to make the definition of life somewhat more accurate.

From this we obtain directly the answer to the much-discussed question as to whether the laws of chemistry and physics are sufficient to explain all biological phenomena. The answer must, from one point of view be yes, from another point of view no. We may say *yes* in so far as all biological phenomena lie within the realm of the possibilities which are given by these sciences. On the other hand, we must say *no* in so far as in this realm of possibilities a much greater complexity is attained by biological facts than it is possible to explain by physics and chemistry.

Perhaps the relationship of which we are speaking will become clearer if we inquire into the analogous relationship which exists between mathematics and physics. It is certain that all physical phenomena are considered under the conception of magnitude, and to this extent physics may be regarded as a part of mathematics. But it is just as sure that it is impossible to gain a complete knowledge of a physical phenomenon by the means afforded by mathematics alone. We can, for example, represent mathematically the physical processes which are connected with the passage of an electric current through a given conductor with that degree of accuracy which is attainable by the present methods of analysis. But we cannot represent mathematically the difference between this process and the conduction of heat through a similar body; for here we meet with new kinds of manifold characters which are not present in mathematics but are dealt with in the first place by physics.

Now since the mechanists look at this matter from one point of view and the vitalists from another, we have at the present day the remarkable phenomenon that in both camps there are highly distinguished and thoughtful investigators who apparently hold opposite views, while there can be no more than one true standpoint; and the members of both camps are known to us as earnest and trustworthy seekers for the truth.

Undoubtedly these general considerations have their value in connection with problems with which we are dealing only in the thorough investigation of the components which serve to distinguish biological objects from chemical objects. We sum up this distinction ordinarily in the word *life*. If we inquire into the parts of this concept which may be recognized and measured we find the following to be true: Living beings in the first place are not stable, but on the contrary are stationary structures. Rapid changes take place in them of such a nature that gain and loss counterbalance one another so that the whole system experiences only slow changes (which are almost all periodic). Since all physical changes may be represented as displacements of different kinds of energy in space and time living organisms are characterized by the fact they keep their condition of energy approximately constant in its nature and amount, while a constant current of various energies flows through their bodies. According to the general laws of energy this can take place only in such a way that the living organism takes up energy of a higher potential and gives it off at a lower potential. In the meantime the energy has been used for those transformations which make up the various activities of life, namely, movements, the production of heat, reproduction, etc.

These characteristics are common not only to living organisms but also to many inorganic structures. A burning candle whose wick is supplied with melted fat at the same rate at which it burns, or a benzine motor which regulates its benzine supply by means of the ball governor in such a way that its velocity remains constant, has exactly the same property as a living organism. Thus we are in the habit of speaking of the "flame of life" or of the "machines of our bodies," but we do not regard these things as living organisms because their existence is not self-maintaining. When the fat is used up, or the benzine is exhausted, the flame goes out and the motor comes to a standstill, for the former cannot produce new fat nor can the latter obtain new benzine.

Still it is possible to conceive of a further regulator which might pump new benzine from a tank into the reservoir of the motor when that which is already there becomes exhausted. But

in the end this also would be used up or some essential part of the machine might become broken so that the stopping of the machine would become inevitable. In order to maintain itself, or others of the same kind, the machine must in the first place be able to go where it can find new benzine, or, on the other hand, it must be able to replace by its own efforts the parts which have been destroyed; or it must, before it is completely broken, be able by itself to create a new machine which will carry on its work. *If such a machine existed we would be obliged to regard it as a living organism.*

Such a conclusion will perhaps seem to you arbitrary. Still among others one very able investigator has expressed the opinion that even if a structure with all the properties and functions of a certain organism could be artificially produced this would nevertheless not be a true living organism. With reference to this it is only necessary to ask the question, how it would be possible to distinguish a true living organism from an artificial one of the same character, assuming that such an organism could be produced. According to the assumption, both organisms are identical in all their properties, that is, in all those which can be recognized. And two things which can not be distinguished from one another must be considered as fundamentally the same.

It will be found that this conclusion is by no means so arbitrary as at first appears. Our conceptions are formed by gathering together the common points of many phenomena and neglecting those factors which are not common to all. In *stable* chemical structures the conception of chemical *substances* includes those objects with identical specific properties, and we are able to form this conception because we can find or produce the individual substances, for example, sulphur, under the most diverse circumstances, and they show always the same specific properties. It is much more difficult to form such conceptions concerning structures in which chemical *changes* are taking place. In order that such structures may be the subject of the formation of a concept they must appear stable at least externally since otherwise we should have no means at all of identification. Since, according to the definition, they are not *stable* they must

at least be *stationary*, but even stationary structures will not lead us to the formation of a concept if they do not always appear in the same form. Structures of this sort which are *physically* stationary are rivers, clouds, and waves; they are essentially characterized by definite special formations for the existence of which conditions are easily and frequently brought together. Structures which are *chemically* stationary occur very much more rarely because the conditions are more difficult to bring about. The only instance which I can present to you is that of *flame*, for the existence of which there are necessary the following factors, namely, the almost omnipresent oxygen, the widespread remains of plants, and the presence of a temperature of about 400° or 500°. And this simple group of conditions very seldom occurs spontaneously, that is, without the intervention of human beings.

It is evident, therefore, that the voluntary formation of such structures as possess in addition to their stationary properties also the power of self-maintenance, that is, the capacity for seeking out the necessary sources of energy must be an extremely rare phenomenon; and only when such a structure is possessed of the property of *reproducing* its kind will the possibility arise of its coming so often under our observation that we may be able to form a corresponding concept. From this point of view the condition of affairs seems to be as follows: These considerations teach us nothing concerning the manner in which the organism may be constructed; but they do indeed teach us that we would never have arrived at the conception of the organism as a stationary chemical structure if this structure did not possess in a special sense the properties of assimilation and reproduction. These properties are those which are always met with in the stationary chemical structures which we call living organisms. They form the new and specific features of biology as opposed to chemistry. In this respect the vitalists are entirely correct.

When, however, according to this point of view it is maintained that we "consequently" shall never be able to explain life, there exists a confusion between a problem of scientific classification and one of experimental investigation. The

explanation of this lies here, as everywhere, in the recognition of real relationships between different series of phenomena. *One* chemical explanation of life we have already discussed. There can be no doubt in the minds of any of us that life without chemical processes in which free energy is available is inconceivable. That which is lacking is the complete analysis of the individual chemical processes of this sort which take place in the living organism. In a general way we know many of these processes, such as the phenomena of oxidation in the tissues, many of the processes of digestion in the intestinal canal, etc. Most of them, however, are still unknown to us. It must as yet remain doubtful whether or not it would be possible to *create* such an organism even if we were possessed of a knowledge of all these processes. We have conceived of and explained many things without having been able to *make* them, and whether the living organism belongs to this group or to the other class of those structures which we can manufacture, can be determined only during a future period of study.

In order to make more clear what I mean I shall call your attention to an illustration. In a space in which there is contained no electricity we can at any time create it; we are able, in other words, to change other forms of energy into electric so that it will include its two factors electrical quantity and electrical potential. On the other hand, we cannot in the same way produce the energy of gravitation but only make it smaller or greater by placing heavy bodies near to, or remote from one another. The cause of this is easy to understand. Electrical quantities always exist in positive and negative forms in equal parts so that their algebraic sum is constantly *zero*. In consequence of this it is possible to produce any desired quantity of electricity without violation of the law of the conservation of capacity, since its total quantity remains zero. The corresponding factor in the energy of gravitation, however, is proportional to the mass and has therefore necessarily a positive value which can be created or changed just as little as the mass itself. At the present time it cannot be predicted with certainty whether or not, in the complete analysis of life, such factors will be discovered which cannot be created. From

the circumstance, however, that life can be voluntarily destroyed it seems to me fairly probable that its voluntary creation is made impossible only by technical and not fundamental difficulties.

What, then, are these difficulties? This question has already been often answered. The difficulties consist in the fact that the most simple organism is a very complex structure, in which numerous different reactions go on side by side, so that they afford one another mutual support in the vital purpose of self-maintenance, etc. For this purpose many things are necessary, especially that the velocities of these reactions should correspond with one another so that they may act together to accomplish the end in view. A machine would break itself to pieces if the movements of all its parts did not succeed one another at equal periods and at the right time. In this instance the problem is solved by having the different parts bound in a fixed relation with one another so that no other motions except those which are intended are possible. Such compulsory combinations of chemical processes are perhaps also present in the living organism; they occur, for example, if in the alimentary canal the materials provided for the necessary reactions are secreted in different parts of the canal and become mixed with previously prepared chyle by being moved along with it in one direction; but with regard to an individual cell such a mechanical solution of the problem is not very probable. Moreover, in the living organism the same task is usually accomplished in very different ways according to conditions.

The further means of accomplishing the same purpose is the *regulation of the velocities of reaction* by means of catalysers or enzymes. The occurrence of such substances in the various parts of the organism was recognized by Berzelius. He, and later on Ludwig, expressed the opinion that the enzymes play a general and very important rôle in the organism. Later investigations have confirmed and extended this idea. There seem to be in reality only a few tissues in which enzymes are not present, and usually a large number of them appear together. While these older investigators in the light of the scientific knowledge of that time, ascribed to enzymes the property of actually causing reactions and consequently of creating certain

materials, we are now in a position to broaden this conception and make it more comprehensive since we know that the enzymes have also the power of regulating the *velocities* of the reactions. That there may exist in a sense a fixed connection between the actions of enzymes is shown, for example, in the later investigations in reference to the germination of seeds. In this process there appear in succession the enzymes of the solution of starch, of oxidation, and of assimilation. The sequence of their appearance, their quantity, and the place in which they appear, are such that the process of development takes place everywhere in the young plant regularly and purposefully.

In this connection there arises a problem of fundamental importance which formerly has not been placed in the realm of biology, since the necessary foundation of general chemistry has been acquired only in late years, namely, the problem of the *time sequence* of processes in the organism. The spatial sequence has been shown to us by the microscope, and I must describe to you, at least the rôle which this instrument has played. Biology has for a long time concerned itself with certain problems of time relationship, especially those connected with embryology. But microscopy in relation to time, the analysis of that which takes place at each instant during life, especially the time microscopy of chemical processes, presents a problem for the solution of which very little evidence has been presented. It is, however, a problem which must be solved if we wish to go earnestly into the solution of the deeper problems which life presents to us.

Where and how these problems are to be attacked will probably be better known by you than by myself. Probably the best method will be to approach them from the most diverse points of view since as a whole they are of inconceivable complexity. Since I myself have never concerned myself with the problems of biology I cannot venture to offer suggestions to the workers in that science. The only thing which I can do is to indicate the general points of view which come to light in the consideration of the general study of science. The problem lying before us of the coördination of a large number

of different phenomena to form a stationary unit scarcely existed in the sciences developed before chemistry; and when such a combination was made it was mainly by means of addition or superposition, that is, the whole series of phenomena related to the point in question was considered as the sum of the individual factors. In this way each of these factors played its own part as though the others were not present. Thus sound waves cross one another in a most complex way without becoming confused, and in the same way two or three or more rows of waves may play over the surface of the sea in different directions and each one retains its own arrangement and its own characteristics. But here one sees already the limitations for the applications of this principle. When a large wave breaks the smaller ones vanish in the disturbance of the surface and are never seen again. This is due to the fact that the waves are stable structures only up to a certain limit; if this limit is overstepped the forms of energy which are present are converted into other forms, and the principle of superposition no longer gives a satisfactory explanation of what happens.

This reaction of which we have just spoken is universally present in organisms. Small changes in one factor of a given condition will bring about proportionately small changes in other factors, but only in the rarest instances will a superposition be evident. When one allows a string to vibrate, a change in temperature will bring about a change of the pitch, but this is simply due to a change in the elasticity of the string, and in the determination of this factor lies the solution of the problem. If the temperature of an organism be raised there occurs not only the corresponding change in the physical properties of the tissues, but at the same time changes are brought about in the velocity of the reactions of all chemical processes which take place in the organism, and indeed each reaction is influenced in a different way. By this means the relation which these reactions hold to one another is changed, and it may happen that through slight influences of this sort in any one place the limit is overstepped within which the organism remains a stationary structure. In such a case death results. It seems to me that there is no doubt that, as I suggested many years ago,

the remarkably accurate and finely adjusted self-regulation of the higher mammals, by which they become converted into thermostats of unusual constancy, has its origin in the necessity of keeping constant the relation which exists between the velocities of the chemical reactions, in order that the processes of life may take a normal course. In this *problem of co-ordination* it seems to me that the most important side of biology lies, namely, the part which has to do with time relations. Here also are present the phenomena which require the formation of concepts of a new sort for their intellectual conquest; concepts which are not necessary in the more general sciences. In these fields, biology will rule by itself, though still not independently of chemistry and physics. It must exert its influence on the other hand, within the limits of empirical possibility laid down by the latter sciences. These limits and conditions, however, the biologist must know and respect, if he wishes to solve his problem. He must be familiar with the ways and means of general chemistry and physics in order to gain an idea of the ways and means of the living organism. This leads us again to the main question on account of which we have undertaken this whole discussion, namely the question: How does the wind now blow upon the sea of science? The answer is as we have already said, towards *synthesis*. The fairest and richest results are to be expected in that place where the various sciences reach out towards one another for mutual help, and you see therefore, how it is that I have been able to predict for our ship a most fortunate voyage. The course which seemed best when observed from the highest standpoint to which I could attain is the same as that which the pilot of this ship has already held for many years. He needs only to steer his accustomed course in order to gain the best which one may hope for. Thus it only remains for us to wish him and his crew a pleasant journey.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 5, pp. 33-37 October 5, 1903

THE LIMITATIONS OF BIOLOGICAL RESEARCH.*

BY

JACQUES LOEB.

The opening of a new laboratory which is preëminently intended for scientific research is an event in which everybody may rejoice and which no one need regret; for it is the function of all scientific research to increase the possibility and value of life. Not only the University but the whole community may feel grateful to those whose generosity has made the laboratory possible. I may be pardoned for adding an expression of my personal gratitude to President Wheeler and Mr. Howard who have shown the greatest consideration for my wishes in regard to the building of the laboratory. I also wish to express my thanks to my colleagues Mr. Fischer and Mr. Cory for the help I have received from them. Last, but not least, our thanks are due to Professor Ostwald. The fact that he has come here to dedicate a new laboratory is perhaps the greatest distinction which could have been bestowed upon the University by the scientific world. But his presence here today means more to us than this. It is being recognized that the economic, hygienic and intellectual progress of a nation depends directly or indirectly upon the results of scientific research. American universities are just taking their first steps towards giving research that place which it has occupied for several generations

* Address delivered at the dedication of the Rudolph Spreckels Physiological Laboratory of the University of California, August 20, 1903.

in European and especially in German universities. If one of the recognized leaders of scientific thought and research in the Old World is willing to interrupt his work and participate in the inauguration of a laboratory on the Pacific Coast, we may be sure that the new departure of American universities has won for this country the sympathy of the best and therefore the real representatives of the European nations.

It is customary to indicate on an occasion like this the general direction and tendencies of the work which will be carried on. I need not dwell upon the importance of physical chemistry for physiological problems as I have often discussed this before. It is realized by all biologists that the source of energy in life phenomena is chemical, while many of the manifestations of life are physical in character. From this alone it is evident that physical chemistry must form the foundation of biology.

It is clear that the more fundamental the problems are which a laboratory undertakes the more it may hope to accomplish, but it is also true that at each period the number of tasks which might be undertaken successfully is limited. What we call the explanation of a phenomenon is according to Mach the possibility of presenting it as an unequivocal function of those variables upon which it depends. Each generation can hope to solve successfully only those scientific problems whose variables it adequately knows and controls.

One of the fundamental problems of biology is to find a definite answer to the question whether or not it is possible to make living matter out of dead matter. No one has thus far succeeded in transforming dead matter into living matter, and no one has thus far observed the spontaneous generation of an organism in nature. In consequence of this fact Arrhenius assumes that living matter has eternally existed, and has been carried through the universe from star to star in the shape of extremely minute particles, such as spores, at or below the limit of microscopic visibility. He has calculated that electrical charges of these particles and radiation pressure suffice to bring about a transmission of living particles from one star to another in a comparatively short time. On the other hand, however,

we are warned by a number of reasons not to be too hasty in assuming the impossibility of abiogenesis. As far as we know the substances found in living organisms are chemically well characterized and can be obtained outside of living organisms. Moreover, the growth of an animal or plant from a microscopic germ to its adult state depends upon a constant transformation of dead matter into living matter. Did this transformation not occur constantly in all living beings no living organism would be left today. As far as the dynamics of this transformation of dead matter into living matter inside an organism is concerned we know that no other specific agencies are involved than enzymes. The action of enzymes, however, does not seem to differ in any way from the action of inorganic catalytic agencies. I do not think it too bold to prophesy that the general physico-chemical character of these enzymes will sooner or later be cleared up. As regards the oxidizing enzymes the goal seems to be already in sight.

In going over in detail all the features of life phenomena we find that many of them can either be imitated in inorganic nature in all their details, or can be controlled unequivocally by physical or chemical means. In fact this is the case to so great an extent that it almost causes surprise that the experimental transformation of dead matter into living has not been accomplished. But we must consider the fact that the peculiar complex of physical conditions which we call the structure of living organisms is absolutely essential for life phenomena. If an organ, a brain for example, be ground to a pulp its function ceases. When the kidney is ground to a pulp it loses the power of transforming benzoic acid and glycocoll into hippuric acid, provided all the cells are destroyed. From this the conclusion was drawn that possibly all the synthetical processes of an organism depend upon the normal structure of the tissues. This certainly goes too far, for we now know that soluble enzymes which can be extracted from the tissues, for example lipase, not only accelerate hydrolytic but also synthetical processes. But it can not be denied that the physico-chemical peculiarities which we call the structure of the living tissue form an essential part in the complex conditions which

determine life phenomena. In regard to the question of abiogenesis the biologist is therefore placed before two possibilities. Biologists will either succeed through a series of discoveries in transforming dead matter into living matter, or they will finally discover that there is as definite a discontinuity between dead matter and living matter as there is between two chemical elements.

Another problem of transformation faces the modern biologist, namely, that of transforming one species into another. If living organisms have arisen from dead matter the first forms of living matter must have been simple in structure, and perhaps not more complicated than spores. If living germs are as old as cosmic matter the transportation of germs from star to star is only imaginable if the germs were extremely small and consequently simple in structure. In either case it is necessary to assume that the present fauna and flora of the earth must have developed from structures of no higher degree of differentiation than, for example, spores. If an investigator were asked today which he considered more difficult, to make a highly organized plant or animal from the spores of a fungus, or to make a spore from dead matter, I am afraid he would hesitate to decide. Yet a majority of investigators have been convinced since the days of Lamarck and Darwin that such an evolution must have occurred. We are better off in regard to our observations concerning evolution than in the question of abiogenesis, for there can be no doubt that hereditary transformations, within narrow limits at least, have occurred. I need only mention the positive observations of DeVries. But all the variations which have thus far actually been observed are extremely slight, so that it is still difficult to grasp an evolution from cosmic spores or protoplasmic material of the simplest structure into such highly developed machines as human beings. As far as I am aware no one has yet found a method of bringing about a rapid variation in animals or plants. I am inclined to believe that this failure is at least partly due to the existence of mechanisms of regulation. We know that our body possesses automatic regulators to keep our temperature constant, that there are automatic regulators for keeping the concentration of

sugar in the blood tolerably constant, etc. Any too sudden and extensive change in an organism probably interferes with these automatic mechanisms, and therefore leads to the destruction of the organism. We again meet with two possibilities: we shall either succeed by a series of continued slight changes in one and the same form in bringing about a large transformation from the original form, or we shall obtain the result that in each form the possibility of evolution is limited, and that, at a certain point, the constancy of a species is reached. Either result if guaranteed by adequate observations will be a welcome discovery.

Before closing allow me to make one additional remark. It is quite common to find that even scientists are inclined to assume that the restrictions or limitations to research in biology are greater than, or of a different order from those in the field of physics or chemistry. This is not true so long as we treat biological problems in the same unbiassed and unprejudiced way in which we deal with the problems of physics and chemistry. The alchemists tried to solve the problem of perpetual motion and of making gold. No one, however, would say that the physicist or chemist is limited in his search for truth because perpetual motion is impossible and the chemical elements are constant. On the contrary, it is fully recognized that the discovery of such constants as the quantity of energy of a closed system or the chemical elements were the most fertile discoveries ever made, and, in fact, are among the pillars on which not only modern physics, chemistry and biology, but indirectly our whole modern civilization rests. Why should we change our attitude when we leave the field of physics and enter the field of biology, and why should we consider it a restriction or limitation of knowledge if it should turn out—which is far from certain, however,—that it is no more possible to transform dead matter into living matter than to transform copper into gold?

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 6, pp. 39-53 November 9, 1903

THE FERTILIZATION OF THE EGG OF THE SEA-URCHIN BY THE SPERM OF THE STARFISH.*

BY

JACQUES LOEB.

I.—Introductory Remarks.

The experiments which I intend to discuss this evening were carried on in Pacific Grove, and it is a source of satisfaction to me that I have a chance to express my thanks on this occasion for the kind hospitality you have repeatedly extended to me at the Hopkins Laboratory.

You are all undoubtedly familiar with the fact that only such animals as are closely related can be hybridized. This fact finds an experimental proof on a large scale in the ocean where the spawning seasons of many forms whose eggs are fertilized in the water must naturally coincide. As the motion of the water carries the sperm long distances, and as, according to Fischer's observations, the sperm of a sea-urchin keeps alive for a week or longer in sea-water it is indeed remarkable that—as far as we know—no hybrids are found in the ocean with the possible exception of very closely related forms.

For years I have tried to find a method by which these apparent barriers might be removed. It was more than the

*Address, delivered before the Sigma Xi Scientific Society at Stanford University, October 13, 1903. A fuller account of the experiments mentioned in this lecture can be found in two papers in Pflüger's Archiv, one of which has appeared (vol. 99, p. 323), while the second at the time this is being printed is still in press.

mere delight in experimental sport which led me to this attempt. I dare say that experimental biology is confronted with two great problems of transformation which it either has to solve successfully, or for which it has to establish beyond doubt the impossibility of a solution. One is the transformation of dead into living matter, the other the transformation of species. The former problem we do not need to consider in this connection. It is doubtlessly true that hereditary transformations of species occur, and it is the merit of de Vries to have shown that these transformations can be directly observed like any other biological phenomenon. But all the changes that have thus far been directly observed in regard to the transformation of species are extremely slight, and, moreover, the cause of the transformation is, as a rule, unknown. However life may have originated on earth we cannot avoid the conclusion that enormous transformations of organisms must have occurred. We cannot hope to gain a deeper and more certain insight into the possibilities for the transformation of species beyond that which we have at present unless we possess methods by which forms varying widely from those existing at present can be produced. It is well known that hybridization is the safest method for bringing about hereditary variations, but if we wish to bring about by this method greater variations than have thus far been accomplished it will first be necessary to find a method by which such forms as are not in close relationship can be hybridized.

Whenever we wish to bring about results experimentally which under natural conditions seem impossible, we must first try whether or not one of the main conditions upon which the phenomenon in question depends is capable of variation. In the case of fertilization of marine animals it had been generally assumed that the only variables were the egg and the spermatozoon. I consequently tried whether it was not possible to break through the barriers which prevent the hybridization of distant forms by modifying the egg or the sperm.

The experiments on artificial parthenogenesis had, as I believe, proved that the spermatozoon carries into the egg two distinct groups of causes or conditions. The one group we may call the developmental group, which causes or accelerates the

process of development; the second we may call the hereditary group, and this group is responsible for the transmission of the paternal qualities to the embryo. The methods of artificial parthenogenesis permit us to imitate the effects of the former but as far as we can judge not of the latter group. It seemed possible that the separate existence of these two groups of conditions in the spermatozoon might be utilized for the purpose of crossing distant forms of animals. It was conceivable that such hybridizations remained futile on account of the fact that the developmental group in the spermatozoon can only act upon the eggs of closely related forms. I therefore thought it possible that if eggs could be caused to develop by the methods of artificial parthenogenesis, the spermatozoon of a distant form might bring about the desired hybridization if it only entered the egg. This method has thus far yielded no results. I am not even certain than an egg after it has been caused to develop parthenogenetically can be entered by a spermatozoon of its own kind.

While in the experiments just mentioned the attempt had been directed toward a modification of the egg another method was tried in which it was intended to modify both egg and spermatozoon. This method was suggested by the experiences of bacteriologists in regard to immunity. It was conceivable that the egg and sperm of distant species were incompatible and that this incompatibility might be diminished if the female of one species were previously treated with the sperm of the distant species, with which the crossing was intended, or vice versa. The experiments were extremely tedious and could naturally not be very numerous. They led to no result. I finally began to realize that in the fertilization of marine animals which deposit the unfertilized eggs in the sea-water the egg and the sperm are not the only variables but that there exists a third group of variables namely the constitution of the sea-water. By varying the constitution of the sea-water I succeeded indeed in bringing about the fertilization of the egg of the sea-urchin *(Strongylocentrotus purpuratus and S. franciscanus)* by the sperm of the starfish *(Asterias ochracea)*, a result which had thus far been vainly attempted.

There were two reasons for selecting the egg of the sea-

urchin for this purpose. It possesses the advantage that it can be caused only by specific means to develop parthenogenetically so that if these means are not applied one may feel sure that the eggs will not develop. With the eggs of annelids or starfish this is altogether different. The eggs of the starfish may even develop in normal seawater without any apparent cause. Delage has found that treatment with CO_2 causes these eggs to develop parthenogenetically, and inasmuch as they produce CO_2 naturally it is quite possible that for a small number of them the CO_2 they produce may suffice to bring about their parthenogenetic development.

Morgan published a number of years ago the statement that he had succeeded in fertilizing a small number of starfish eggs by the sperm of sea-urchins. It had been pointed out before that in Morgan's experiments the eggs had developed parthenogenetically. Von Dungern has recently proved that the starfish eggs contain a substance that is poisonous for the sperm of the sea-urchin, and which renders the fertilization of starfish eggs by the sperm of the sea-urchin absolutely impossible. It is obvious enough that starfish eggs are not the right kind of material for experiments on hybridization and the same may be said in regard to the eggs of many annelids.

The second reason why the egg of the sea-urchin was chosen for these experiments was the fact that this egg indicates the entrance of a spermatozoon by the formation or by the suddenly rendering visible of a thick membrane, the so-called membrane of fertilization. This membrane is not formed when the egg is caused to develop parthenogenetically.

II.—The Solutions in Which the Eggs of Sea-urchins (*Strongylocentrotus*) Can Be Fertilized with the Sperm of Their Own Species.

It has generally been taken for granted that for the process of fertilization it is sufficient that the eggs and spermatozoa be brought together. I have found, however, that for the eggs of sea-urchins, at least, and probably for all the eggs that are fertilized

in the ocean, certain substances contained in the sea water are necessary while other constituents of the sea-water are not required.

If one tries to ascertain which substances are necessary for the fertilization of the eggs of an animal by the sperm of its own species special precautions must be taken. Before the eggs and sperm are brought together both must be washed separately for several minutes in those solutions in which the fertilization is to be tried. I proceeded as follows: 50 cc. of the solution in which the fertilization was to be tried were put into two separate dishes; into one dish was put the sperm, into the other the eggs. In order to contaminate the solution as little as possible with sea-water only 1 or 2 drops of eggs, and just as little, or still less, sperm was added. Eggs as well as sperm remained 3 to 5 minutes in these solutions. Both solutions were then mixed and thus sperm and eggs brought together. If one proceeds in this way one can easily see that fertilization is only possible in definite solutions. But if the eggs and sperm are not washed before being mixed, contradictory results are obtained, inasmuch as in that case fertilization is occasionally possible in every solution. In reality, however, the fertilization does not, in this case, occur in the solution, but the spermatozoon and eggs are surrounded by a surface film of sea-water or liquid from the ovary or testes and the result is the same as if for such eggs and spermatozoa the solution were not present at all.

A second circumstance to be considered in these experiments is the concentration of the sperm. It is necessary to work with a definite concentration of sperm in order to obtain comparable results. I proceeded as follows: I first found out the minimal quantity of sperm which suffices for the instantaneous fertilization of 2 drops of eggs in 100 c.c. of sea-water. If a slighty opaque suspension of sperm be used, the addition of 2 or 3 drops of such a suspension is as a rule sufficient to produce the fertilization membrane in all the eggs in 100 c.c. of sea-water within five minutes or even less. If this minimal concentration of sperm for normal sea-water has once been found one has to work with the same concentration in all the other experiments in which the same eggs and the same sperm are used. Of course one must

consider that a number of spermatozoa soon die, and that a time occurs when the concentration of sperm which sufficed at the beginning is no longer adequate. In artificial solutions which are not favorable to fertilization it is found that with an increase in the concentration of the sperm the percentage of eggs which are fertilized increases. This may be due to the fact that in such artificial solutions a large percentage of the spermatozoa is rendered unfit for fertilization and not enough spermatozoa are left to bring about the rapid fertilization of all the eggs. It is, however, possible that the sperm also modifies the chemical constitution of the solution, either by substances diffusing from the spermatazoon into the surrounding solution or perhaps by secretions from the sexual glands being carried with the sperm into the solutions. I have tried to see whether the addition of an extract of sperm increases the fertilizing power of spermatozoa under such conditions, but thus far without any positive results. Which substances contained in the sea-water are necessary for the fertilization of the eggs of sea-urchins by sperm of their own species?

According to van't Hoff sea-water is a solution in which the constituents are present in the following molecular concentration: NaCl 100, KCl 2.2, $MgCl_2$ 7.8, $MgSO_4$ 3.8. To this is to be added Ca; in our experiments 2 $CaCl_2$ were added. As a solution of these salts will be used quite frequently we shall call it briefly the van't Hoff solution. In our experiments we used $\frac{m}{2}$ *i.e.* half grammolecular solutions corresponding with the concentration of the sea-water.

I was surprised to find that in the van't Hoff solutions which I used the egg of *Strongylocentrotus* could, as a rule, not be fertilized by sperm of their own species, although the spermatazoa were very motile and surrounded the eggs densely. When to 100 c.c. of such a solution 0.1 c.c. of an $\frac{n}{10}$ solution of NaOH was added all, or most of the eggs were fertilized. If 0.15 or 0.2 c.c. of $\frac{n}{10}$ NaOH are added to 100 c.c. of the van't Hoff solution fewer or no eggs were fertilized, and upon the addition of as much as 0.3 c.c. of an $\frac{n}{10}$ NaOH solution generally no egg was fertilized, provided that a minimal concentration of sperm which is sufficient for the fertilization in sea-water was used. It

seemed at first as though these facts indicated the necessity of free hydroxyl ions for fertilization, but tests of the van't Hoff solutions which were afterwards made showed that these solutions were slightly acid and that 100 c.c. of the solution required about 0.04 c.c. of an $\frac{n}{10}$ solution of sodium hydroxide to become neutral to phenolphthaleïne. The minimal quantity of $\frac{n}{10}$ NaOH which I had to add to 100 c.c. of the van't Hoff solution in order to bring about the fertilization of the eggs was about 0.08 c.c. But it is to be considered that in contact with air the carbon dioxide contained therein very rapidly neutralises the alkali in such a solution, and I think we must therefore conclude that the alkali required in these experiments only served to render the van't Hoff solution neutral, and that hydroxyl ions are not necessary for the fertilization of the sea-urchin egg with sperm of its own kind.

The correctness of this conclusion was proved beyond doubt by the fact that normal sea-water in which the eggs are naturally fertilized by sperm of their own species is a neutral solution. It had been generally accepted that sea-water is an alkaline solution. The same statement had been made in regard to the blood, but recent investigations of Friedenthal, Fränkel, and Farkas have shown that the concentration of the hydroxyl ions in the blood is no greater than in distilled water. Tests which I made with phenolthaleïne showed that normal sea-water must also be considered as a practically neutral solution. Dr. Cottrell was kind enough to make, at my suggestion, a determination of the concentration of the hydrogen ions contained in the sea-water of the Bay of San Francisco with gas batteries, and confirmed the result that I had obtained with phenolthaleïne, namely, that sea-water must be considered as a neutral solution. The concentration of the hydrogen ions which he found in the sea-water is even one power of ten higher than that found in distilled water. It is in harmony with this result that the addition of a slight amount of bicarbonate of sodium to the van't Hoff solution has the same effect upon the fertilization of the eggs of the sea-urchin by sperm of its own species as the addition of a trace of alkali. It is obvious that the bicarbonate could not make the van't Hoff solution alkaline, but could neutralize the free acid which was contained in it.

We can therefore say, as the outcome of all the experiments, that the fertilization of the sea-urchin egg by sperm of its own species succeeds best in a perfectly neutral solution like sea-water.

As far as the other constituents of the sea-water, aside from H and HO ions, are concerned, I found that fertilization was possible in any solutions which contained sodium chloride and calcium chloride (provided the solution was neutral), and that the addition of any other of the constituents of the sea-water, say potassium or magnesium, did not make the solution more favorable. In solutions which did not contain any calcium but contained all the other constituents of the sea-water fertilization was absolutely impossible. The same was true for sodium. But in neutral solutions of NaCl and $CaCl_2$ without the other constituents of the sea-water fertilization occurred just as rapidly and completely as in normal sea-water. The unexpected result was therefore obtained, that in the fertilization of the sea-urchin egg with the sperm of its own kind, calcium and sodium salts or ions play an important rôle, while the other ions and salts contained in the sea-water are insignificant and cannot act as a substitute for calcium or sodium. It was also impossible to substitute for the sodium chloride solution an isosmotic solution of cane sugar or of lithium chloride.

III.—On Solutions in Which the Eggs of Sea-urchins Can Be Fertilized with the Sperm of the Starfish.

We have seen that normal sea-water has a neutral reaction and that in sea-water as well as in artificial solutions, when they contain sodium chloride and calcium chloride in the right proportion, and if they are neutral, the eggs of sea-urchins can be fertilized by sperm of their own kind. The fertilization of the same eggs by sperm of the starfish is, however, impossible in such solutions. By a slight change in the nature of these solutions the hybridization can be accomplished; it is only necessary to give the solutions a slight but definite degree of alkalinity. When about 0.3 to 0.4 c.c. of an $\frac{n}{10}$ sodium hydroxide solution are added to 100 c.c. of the van't Hoff solution,

about 50 to 80 per cent. of the sea-urchin eggs may be fertilized in a rather short time upon the addition of living sperm of a starfish. In this case the eggs form a membrane of fertilization and at the proper time segment and develop into swimming larvae. If one adds a little less sodium hydroxide, for example about 0.2 c.c. instead of 0.3 or 0.4 c.c., very few or no eggs are fertilized upon the addition of the sperm of the starfish. If one adds only 0.1 c.c. of sodium hydroxide to 100 c.c. of a van't Hoff solution, not a single egg is, as a rule, fertilized by the sperm of the starfish. If more than 0.4 c.c. of a $\frac{n}{10}$ normal sodium hydroxide solution to 100 c.c. of the van't Hoff solution is added as a rule no eggs are fertilized. As far as the other constituents of the van't Hoff solution are concerned only sodium and calcium are essential for the fertilization of the sea-urchin egg by the sperm of the starfish. The presence or absence of the other constituents of the sea-water does not affect the result, with this difference, however, that the subsequent development is, of course, the better the more closely the artificial solution resembles the sea-water in its constitution. A number of objections are now to be considered. What guarantee have we, after all, that in this case the development of the sea-urchin egg is due to the starfish sperm and not to some other cause? Among the other causes that might possibly be considered we have first to think of the contamination of the eggs with sperm of their own kind. This possibility was excluded by the fact that each series of experiments was accompanied by control experiments in which part of the sea-urchin eggs of the same female that were used for the experiment were left in normal sea-water without the addition of any sperm. Not an egg developed in this case, showing that the eggs were not contaminated with sperm of their own kind. Moreover, we shall see later that in the very solutions in which the eggs of the sea-urchin can be fertilized by the sperm of a starfish they cannot, or only with difficulty, be fertilized by sperm of their own species. A second objection to be considered is the following: The development of the sea-urchin eggs in these experiments was perhaps not due to the sperm of the starfish but occurred parthenogenetically. I had to think of the possibility that the solutions in which the eggs

of the sea-urchins developed upon the addition of the sperm of the starfish were of such a character as to cause artificial parthenogenesis of the egg of the sea-urchin. This possibility was excluded by the fact (which I confirmed in each series of experiments) that when the eggs of sea-urchins were put into the solutions favorable for hybridization, but without the addition of sperm, not an egg formed a membrane of fertilization or segmented. In some cases I continued the observation of such eggs as long as six days without noticing any of the changes indicative of development. Another possibility was that artificial parthenogenesis might be caused by the agitation connected with adding the sperm to the eggs or by some other mechanical or thermal conditions in these experiments. I excluded this possibility by treating the control eggs in every point like the eggs to which sperm had been added. The eggs that were kept in the artificial solutions without sperm were agitated and stirred in the same way as those to which sperm was added, but only those eggs formed a membrane of fertilization and developed to which the starfish sperm was added. Finally the possibility existed that it was not the living spermatazoön at all that caused this development, but some substance introduced into the sea-water with the sperm of the starfish. In order to test this possibility starfish sperm was heated to a temperature of between 45° and 60° and kept at this temperature for some time, to kill the spermatozoa. When sperm whose spermatazoa were dead was added to the eggs of the sea-urchin contained in the proper solution not an egg formed a membrane of fertilization or developed. From all these facts it seems to me we must conclude that the formation of the membrane of fertilization, and the subsequent development of the sea-urchin eggs upon the addition of starfish sperm in these experiments was caused by the living spermatozoa of the starfish. The question now arises: Why is it that the sperm of starfish can cause the development of sea-urchin eggs when the latter are in a solution of an alkalinity of about $\frac{3}{10000}$ normal, while this cannot be accomplished in a neutral solution or a solution of lower or higher alkalinity? This question I am as yet unable to answer definitely. When eggs alone were put for various lengths

of time into this alkaline solution and then placed in sea-water which contained the sperm of a starfish no egg was fertilized. When starfish sperm was kept for various periods of time in the alkaline solution and then brought into sea-water which contained the eggs of the sea-urchin no fertilization occurred. It is therefore obvious that fertilization can only be accomplished while egg and sperm are in the alkaline solution. If, however, the eggs are once fertilized they may be taken out from this artificial solution and put back into normal sea-water without their development being stopped. Hence the alkaline reaction is only necessary for that brief interval during which the sperm enters the egg and, perhaps, the first minutes after this. It is not impossible that the whole effect of the alkali is simply to bring about a slight physical change in the condition of the protoplasm or the micropyle of the egg, or the surface of the spermatazoön which facilitates the entrance of the latter into the egg. There are, however, other possibilities upon the discussion of which we cannot enter here.

I was at first inclined to believe that the alkaline solution which was required for the fertilization of the sea-urchin egg by the sperm of the starfish increased the motile power of the spermatazoa, and that in this way it helped to bring about the fertilization, but I was finally compelled, in view of my direct observations, to abandon this idea for I found that in a van't Hoff solution to which bicarbonate of sodium had been added the motility of the spermatazoa of the starfish was greater than that observed in any other solution, and yet in such a solution the eggs of the sea-urchin could not be fertilized with the sperm of the starfish. On the other hand I gained the impression that the very alkaline solution in which the sea-urchin egg could be fertilized by the sperm of the starfish rather diminished the motile power of the spermatazoa; they come sooner to rest in such a solution than if only bicarbonate of sodium is added.

It became further necessary to settle the question whether it was not possible, at least under certain conditions, to bring about the fertilization of the sea-urchin egg in normal sea-water. My experience in that respect was rather remarkable. Whenever I used sea-water that came from the large

tank at Pacific Grove it was not possible to fertilize an egg of the sea-urchin with the sperm of the starfish, but when I used water that was taken directly in a jar from a certain spot at the beach I occasionally observed that a few eggs could be fertilized. This water was of course heated in order to kill the spermatozoa that might have been contained in it. I was at first inclined to think that perhaps the water circulating in the pipes of the laboratory contained some metallic salts which acted as poisons, but this argument lost in force by the fact that in the same water the eggs of the sea-urchin could be fertilized quite readily with sperm of their own kind. This showed that the toxicity of the water, if there was any, could not be of a very high order. When later on in Bolinas I repeated the same experiments I found that water taken directly from the ocean in Bolinas did *not* allow the fertilization of the sea-urchin eggs with starfish sperm. I am not yet ready to explain these differences definitely, but I may state the following facts: The water at Bolinas was taken from a region which was for a long distance practically free from green plants, while the water I took directly from the bay at Pacific Grove came from a region where algae were abundant, and so close to the surface that their assimilating action must have been rather powerful. Now I have found recently that while sea-water in the absence of plants has a neutral, or faintly acid reaction, it becomes faintly alkaline when algae are put into it and the latter are exposed to the light. Now the alkaline reaction is as we have seen the essential condition for the fertilization of the eggs of the sea-urchin with the sperm of the starfish. I intend to continue these tests of the sea-water. They are certainly remarkable in that they show that the sea-water can not after all be considered as a constant entity, but that it shows variations in regard to that constituent which is a most powerful substance for life phenomena, namely, the HO ions.

I tried to ascertain whether the addition of sodium carbonate to sea-water would not render the latter more favorable for the fertilization of the eggs of sea-urchins by the sperm of the starfish, and although the experiments have not been carried far enough to warrant final judgment it looks as if such were the case.

In all experiments in which the eggs of the sea-urchin were fertilized with the sperm of the starfish the concentration of the sperm had to be considerably higher than in the case of the fertilization of the same eggs with sperm of their own kind. I am inclined to ascribe this peculiarity to the possibility that at best only a very small percentage of the spermatozoa of a starfish possess the quality necessary to fertilize the eggs of a sea-urchin. Hence it is necessary to add a large excess of starfish sperm if one wishes to supply enough spermatozoa which are capable of entering the eggs of a sea-urchin.

IV.—On the Relative Immunity of the Eggs of Sea-urchins Towards the Sperm of Starfish and Towards Sperm of Their Own Kind.

I have repeatedly mentioned the fact that the solutions in which the eggs of the sea-urchin can best be fertilized by sperm of their own kind are not identical with the solutions in which they can be best fertilized by the sperm of the starfish. The facts in this case seem to bear upon the problem of infection and immunity towards infection, and for that reason I gave it special attention. In neutral solutions such as normal sea-water, a neutral van't Hoff solution, or a mixture of sodium chloride and calcium chloride solutions in the right proportions, the eggs of the sea-urchin are fertilized within a few minutes by sperm of their own kind. Not an egg is fertilized under such conditions by the sperm of the starfish. On the other hand, in a van't Hoff solution which is made 0.0003 to 0.0004 normal alkaline, 50 to 80 per cent. of the eggs of the sea-urchin can be fertilized in a short time by the sperm of the starfish, while in the same solution none or but few eggs of the sea-urchin can be fertilized by sperm of their own kind. When we transform this statement into the terminology used by the bacteriologist we may say that while in the neutral solutions the sea-urchin egg can be fertilized by sperm of its own kind it is immune to the infection by starfish sperm; while in 0.0003 or 0.0004 normal solutions of NaOH the eggs of the

sea-urchin are practically immune to the sperm of their own kind while they are readily infected by sperm of the starfish. Of course whoever makes such experiments must realize that when an alkaline solution is exposed to the air its degree of alkalinity diminishes on account of the entrance of the carbon dioxide from the air, so that it is evident that these figures only apply to fresh solutions. If one takes a van't Hoff solution and adds sodium bicarbonate (which does not contain any sodium carbonate) the eggs of the sea-urchin are immune in such a solution against the sperm of the starfish, while they can be fertilized by sperm of their own kind. But if one adds a small but definite amount of sodium carbonate instead of bicarbonate, the eggs of sea-urchin become immune to the sperm of their own kind, while they are readily infected by sperm of the starfish.

The fact that such slight variations in the concentration of hydroxyl ions so radically alter the susceptibility of the eggs for various kinds of sperm may possibly apply to cases of diminished or increased resistance of cells or tissues to infection by micro-organisms. The variations in the degree of alkalinity observed in these experiments are within the limits in which they may occur in any cell or tissue.

V.—The Development of the Eggs of the Sea-urchin After Fertilization by the Sperm of the Starfish.

It was of interest to ascertain whether the larvæ which are produced by the fertilization of the sea-urchin egg with starfish sperm show only the characteristics of the larvæ of the sea-urchin, or whether they show also the characteristics of the larvæ of the starfish. It is well known that in cases of hybridization the first stages of development are generally determined by the nature of the egg, and in this case too, the segmentation of the eggs of the sea-urchin which developed upon the addition of starfish sperm differed, as far as I can judge, very little from that of the eggs fertilized by sperm of their own kind. The main difference that was to be expected in this case was in regard to the skeleton. The pure larva of the sea-urchin forms a skeleton

after it has reached the gastrula stage, while the larva of the starfish does not form a skeleton in the corresponding stage. I was interested to find out whether or not the sea-urchin eggs fertilized by the sperm of the starfish were able to form a skeleton. I am not yet ready to give a definite answer in regard to the result. It is certain that a beginning of a skeleton showed itself in a small number of larvæ, but it is also certain that the formation of the skeleton was retarded if compared with the formation of the skeleton of pure larvæ, and that even at the best was never complete. This looks indeed as if these larvæ behaved like a real cross between the sea-urchin and the starfish, but before I am willing to admit this I have the following objections to raise: Whenever we succeed in the case of hybridization in introducing a positive characteristic of one of the parents into a larva we can be tolerably certain that this is the transmission of a hereditary character, but when we are dealing with a negative characteristic, as in this case, when a skeleton is poorly developed or not developed at all, we cannot be equally certain, because a deficiency can be produced by a mere disturbance in the development of the embryo, and cannot be considered as a sure indication of the transmission of the hereditary characters of one of the parents. I intend to continue these experiments and hope to be able at the end of the next season to give a more definite answer to this and other questions.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 7, pp. 55–69 November 30, 1903

ON THE RELATIVE TOXICITY OF DISTILLED WATER, SUGAR SOLUTIONS, AND SOLUTIONS OF THE VARIOUS CONSTITUENTS OF THE SEA-WATER FOR MARINE ANIMALS.[1]

BY

JACQUES LOEB.

1. v. Fürth mentions in his excellent book on the "Comparative Chemical Physiology of the Lower Animals,"[2] the statement of Plateau that the deleterious action of fresh water on marine crustaceans "cannot possibly be due to the difference in concentration between fresh- and sea-water." Plateau founds his idea on the observation that a sugar solution of the same *specific gravity* as sea-water proves likewise fatal for the animals. v. Fürth states correctly that in consequence of the high molecular weight of cane sugar the sugar solution used by Plateau possessed a considerably smaller osmotic pressure than the sea-water. v. Fürth regards Plateau's view as erroneous and it is certain that the researches of the latter author are insufficient as a basis for his theory. The conception of osmotic pressure was, at that time, not yet established. Plateau's work is not accessible to me. I had, before I obtained v. Fürth's book, already made investigations on the cause of the poisonous action of distilled water on

[1] Translated from Pflüger's Archiv, Vol, 97, p, 394, 1903, by J. B. MacCallum.
[2] S. 620. Jena. 1903.

marine animals which led to the unexpected result, that certain animals die as quickly in sugar solutions isosmotic with sea-water as in distilled water, and that therefore the difference between the total osmotic pressure of sea-water and distilled water cannot be the cause of the poisonous action of distilled water.

The researches were carried out on a marine crustacean, Gammarus, possibly an animal of the same species as that with which Plateau worked. In my experiments it was determined how long the respiratory movements of the animals persisted in distilled water or in other solutions. The respiratory movements, as is well known, are carried out in these animals by means of the abdominal gills.

The analysis of the sea water of the bay of San Francisco in which the material for my investigations was collected gave a content in salt of about 2.2 gms. per 100 c.c. of sea water.[1] According to van't Hoff the relation of the constituent parts of the sea-water is about the same in all seas with the exception of the proportion of calcium salts which undergoes variations. The composition of this water is, expressed in the concentration of molecules, 100 NaCl, 2.2 KCl, 7.8 $MgCl_2$, 3.8 $MgSO_4$.

A preliminary estimation of the calcium contents of the sea water of the bay showed that to 100 NaCl there was about 1 $CaCl_2$.

If the species of Gammarus, to which these researches refer and which is not yet exactly determined, be transferred suddenly from sea-water[2] into distilled water the breathing of the animals ceases (at a temperature of about 20° C.) in about half an hour; sometimes still sooner. If the animals are brought, directly after the cessation of breathing, back into the sea-water they are able to recover. If about ten minutes or longer elapses no recovery takes place. The sojourn in distilled water causes changes in the abdominal ganglia of the animals which become in a short time irreversible and from this the death of the animal results. By what means does the distilled water bring about this action? One will be disposed to point out,—and I myself

[1] The salt content in the harbor of San Francisco changes with the tides.

[2] The Gammari were lifted on a sieve and the latter was freed as much as possible from sea water by means of filter paper before the animals were placed in distilled water or in any other solution.

at first was of the opinion—that the entrance of the distilled water into the tissues of the animal was responsible for the fatal action. This idea, however, is restricted by the following investigation, if not contradicted:

2. If the animals be transferred to a pure sugar solution of about the same osmotic pressure as that of the sea-water in which they live, the respiratory movements cease in about as short a time as in distilled water, namely, in about half an hour. The osmotic pressure of sea water lies between that of a $\frac{3}{8}$ m and $\frac{m}{2}$ sugar solution.

A sugar solution almost isosmotic with sea-water is just as poisonous as distilled water.

If a series of sugar solutions be prepared from the concentration $\frac{5}{8}$ m to 0 it is found that the duration of life of the Gammari is about the same in all of these solutions and varies between thirty, forty-five, or sixty minutes. *The toxicity of the sugar solution is in large part independent of its osmotic pressure, and the duration of life of Gammarus in such a solution is of about the same length as the duration of life in distilled water.* The experiment cited in Table I may serve as an illustration.

TABLE I.

Nature of solution.	Duration of respiratory movements of the animal.
100 ccm. $\frac{5}{8}$ m cane sugar	30 minutes.
100 " $\frac{4}{8}$ " " "	38 "
100 " $\frac{3}{8}$ " " "	38 "
100 " $\frac{5}{16}$ " " "	38 "
100 " $\frac{m}{8}$ " "	45 "
100 " $\frac{m}{16}$ " "	45 "
100 " Distilled water	30 "

The same facts may be demonstrated in still another way. If the concentration of the sea-water be diminished by the addition of distilled water the duration of life of the animals is shortened to a greater extent the greater the dilution of the sea-water. Is the duration of life of the animals lessened as much

if the sea-water be diluted with an isosmotic solution of sugar? This is found to be the case.

If the animals be placed in sea water whose concentration is diminished by one-half by means of a mixture of equal parts of sea-water and distilled water, the duration of their life is scarcely influenced. They live for days in such a solution, behave quite normally, and fall finally victims to bacteria or die from other secondary causes which have nothing to do with the dilution of the sea-water, and which are also present in normal sea-water. If the sea-water itself be diluted almost four times—in the experiments under consideration 70 parts of distilled water were added to 30 parts of sea-water—the animals still live more than 48 hours. This duration of life might probably be considerably increased if the animals were contained in well ventilated aquaria instead of in the small vessels used in these experiments which contained only 100 ccm. of water. If kept in the same vessels and in normal sea-water they frequently die also in two days. In sea-water diluted ten times (10 ccm. sea-water and 90 ccm. distilled water) they live generally from 8 to 20 hours; after this the duration of life decreases very quickly with further progressive dilution. In sea-water diluted 40 times they die almost as quickly as in distilled water. With dilutions of twenty times they may live about two hours. For the dependence of the duration of life of these animals on the degree of dilution of sea water by distilled water it is characteristic that *up to a certain limit of dilution the duration of life decreases but little with additional dilution; that then, however, from a certain limit on, the decrease in the duration of life takes place very rapidly.* I believe that this fact is of theoretical and practical significance.

If sea-water be diluted with a $\frac{3}{8}$ m or $\frac{1}{4}$ m solution of cane sugar instead of with distilled water there is obtained a curve of the duration of life of the same form as in the case of dilution with distilled water. For equal degrees of dilution, however, the duration of life in the dilution with sugar solutions is never longer than in the dilution with distilled water, but in general somewhat shorter. This seems to show that sugar is not an entirely indifferent substance for the animal. This is further proven by the fact that if the seaw-ater be diluted with a $\frac{m}{16}$ or $\frac{m}{8}$

sugar solution the duration of the life of the animal is a trifle longer than with equal dilutions of the sea-water by a $\frac{3}{8}$ m or $\frac{m}{4}$ sugar solution.

We must draw the following conclusions from these experiments: Either the distilled water acts in a toxic way by entering into the tissues, in which case, however, the tissues of the animal must be just as permeable for the sugar as for the distilled water; or the toxic action of the distilled water for Gammarus is dependent especially on the fact that certain salts or ions diffuse from the animals into the distilled water. With this assumption it would be clear, without further discussion, why an isosmotic or a dilute sugar solution is just as poisonous for the animal as distilled water. Whether the permeability of the superficial layers of the cells is changed or not cannot be decided by direct observation.

3. We must ask the question as to what salts might diffuse from the tissues of Gammarus in such a short time and in such quantities that death results. A direct answer, by analysis of the distilled water before and after it has been inhabited by Gammarus for a short time, presents this technical difficulty, namely, that it is impossible to isolate those small particles of seaw-ater which are found in the angles and joints of the many segments of the animal. Since it is generally admitted that with death a gradual increase in the permeability of the membranes of living structures is brought about, it would be difficult to determine what part of the salt found in the distilled water *caused* the death of the animal, or what salts diffused out *in consequence of the death*. A second and much simpler way is to determine what salts must be added to the distilled water in order to remove its toxicity. When salts enter from the surrounding medium into the tissues of Gammarus and vice versa, it is to be assumed that an equilibrium exists between the osmotic partial pressure of all the ions in sea-water and in the tissues of Gammarus. *If we place Gammarus in pure sodium chloride solution of about the same osmotic pressure as that of sea-water—about $\frac{3}{8}$ m NaCl solution—the respiratory movements cease in about half an hour.*[1] About the same results are obtained if instead of a $\frac{3}{8}$ m a $\frac{m}{4}$ or $\frac{m}{8}$ sodium chloride solution is used.[2] *A pure sodium chloride solution is*

about as poisonous for Gammarus as distilled water. Since in a solution of this sort, if it possesses the osmotic pressure of the sodium chloride solution contained in the sea-water, the sodium and chlorine ions cannot diffuse from the animal into the outer solutions, (and the same is true also for the sodium chloride molecules which are not dissociated) it must be expected that the toxicity of distilled water depends on the fact that all or some of the remaining constituents contained in the sea-water diffuse out from the animal. If to distilled water or to a $\frac{3}{8}$ m sugar solution there be added all the remaining salts of sea-water in the concentration in which they are contained in sea-water—that is, to 100 ccm. of distilled water or sugar solution, 7.8 ccm. of $\frac{3}{8}$ m $MgCl_2$, 3.8 ccm. of $\frac{3}{8}$ m $MgSO_4$, 2.2 ccm. of $\frac{3}{8}$ m KCl, 1 ccm. of $\frac{3}{8}$ m $CaCl_2$—the animals die not only as quickly as in distilled water or sugar solution, *but more rapidly.* The fact that distilled water increases in toxicity if all the salts contained in sea-water, with the exception of sodium chloride, be added to it in the concentration in which they are contained in sea-water, is a very striking demonstration experiment.

This result was not unexpected since I had been able, on the basis of work previously published on the antagonistic action of ions, to predict it. I had already arrived in general at the idea that a pure sodium chloride solution of the concentration in which it is contained in the sea-water is poisonous for marine animals; and that it is possible for marine animals to live in this medium only because other salts present in the sea-water counteract the effect of the sodium chloride.[3] For the Fundulus eggs and medusæ it is essentially the bivalent Ca ions which counteract the poisonous effect of the sodium chloride. For the eggs of Arbacia the K ions must be present also.[4] I sought now to determine what ions are necessary to prevent the poisonous action of NaCl in sea-water in the case of Gammarus and found that if 1 ccm. of $\frac{3}{8}$ m $CaCl_2$ be added to 100 ccm. of a $\frac{3}{8}$ m NaCl solution

[1] Twitching of the muscles in the whole body may, however, exist for a long time.

[2] Mixtures of $\frac{3}{8}$ m NaCl solutions with a $\frac{3}{8}$ m solution of cane sugar prove just as poisonous as pure sodium chloride solutions of the same degree of dilution.

[3] Loeb; American Journal of Physiology. Vol. III, pp. 327, 383, and 434. 1900.

[4] Loeb, Archiv für die ges. Physiologie Bd. 88, S 68. 1901.

the animals die even more rapidly than in a pure NaCl solution. The addition of 2.2 ccm. KCl to 100 ccm. $\frac{3}{8}$ m NaCl lessens the toxic action of the latter solution a little. If, however, 2.2 ccm. of $\frac{3}{8}$ m KCl and 1 ccm. of $\frac{3}{8}$ m $CaCl_2$ be added to 100 ccm. $\frac{3}{8}$ m NaCl the animals live in such a solution almost as long as in normal sea-water. The addition of $MgCl_2$ has the effect of increasing the duration of life somewhat; but the further addition of $PO_4^{\equiv}$, $CO_3^{=}$, $SO_4^{=}$, and HO-ions improved in no way the results obtained in the previous experiments. In such solutions of NaCl, KCl, $CaCl_2$ with $MgCl_2$ I have kept Gammari alive for as long as a week.

The question now arises whether other metals can be substituted for potassium and calcium. I have been able to find up to the present time no substitute for potassium, but in a mixture of $\frac{3}{8}$ m solutions of NaCl, KCl. and $MgCl_2$ in the proportion in which these substances are found in sea-water the Gammari may live 24 hours or longer. The mixture is, however, not so favorable as that of NaCl, KCl, and $CaCl_2$. $CaCl_2$ cannot be replaced by the chloride of Ba, Co, or Mn. Strontium I have not investigated. It might be possible to replace Ca by Sr. I am inclined to assume that it is the ion of the bivalent metal which removes the poisonous action of NaCl in this case, and that the potassium ions play only the rôle of a necessary addition. As I have often stated in earlier times, every electrolyte, if it is alone in solution in sufficient concentration, endangers life, or in other words is poisonous. This poisonous action can be counteracted by certain other ions. Calcium ions in a very small concentration counteract the poisonous action of a much more concentrated sodium chloride solution (in the case of Fundulus 1000 times more concentrated).[1] This antagonism between sodium chloride and calcium ions may be due to this that sodium chloride and calcium ions influence one and the same substance in the tissues in unequal or perhaps opposite ways (for example, lecithin according to Koch's observations). It is, however, possible that other constituents in the tissues are influenced by only one of the two electrolytes, NaCl and $CaCl_2$; or that NaCl and $CaCl_2$ do not

[1] Loeb; American Journal of Physiology. Vol. 6, p. 411. 1902.
Loeb and Gies; Archiv für die ges Physiologie, Bd. 93, s. 246. 1902.

compensate one another completely in their action on other constituents in the tissues. In this case a compensation for the excessive poisonous action may be caused by the addition of a further electrolyte or ion. If this takes place in the case of Gammarus potassium would play this rôle. These considerations bring us again to the idea that sea-water like our blood is a physiologically balanced solution, i. e. a solution in which all the poisonous actions, which each individual group of ions or molecules would have on the tissues if they were alone in solution compensate one another. In this compensation the valency of the kations plays an important rôle. For the valency of the anions such a function has not yet been discovered; it may, however, exist for certain constituents of the protoplasm and may yet be found. In addition to the valency, however, the specific chemical actions of each metal must be considered. In the case of Fundulus eggs these chemical influences are small in comparison with the influence of valency. In the case of Gammarus they are much more noticeable. This difference will, I believe, prove of significance.

The statement which has been made above that the three metallic ions, Na, K, and Ca, must be considered essential for the respiratory movements of Gammarus, and that the anions are of secondary importance for the removal of the poisonous effect of the solution, is based not on the single fact that a sodium chloride solution may be made harmless by the addition of the chlorides of potassium, and calcium, (and magnesium). I have made experiments to determine whether a mixture of 100 ccm. $\frac{3}{8}$ m NaCl + 2.2 $\frac{3}{8}$ m KCl (which soon kills the animals) may be made harmless by the addition of various quantities of sodium salts possessing anions of a higher valency. In these experiments Na_2SO_4, Na_2HPO_4, sodium oxalate, and sodium citrate were employed. The result was absolutely negative; in no case did the addition of one of these salts increase the duration of life of the animals. The citrate and oxalate shortened the duration of life a little even in the small quantities in which they were used, but probably because they diminished the concentration of the Ca ions in the tissue.

In an earlier paper I have discussed the question as to

whether the electrical charges of ions are responsible for the apparently stimulating action of salt solutions upon nerves and muscles.[1] The fact that the respiratory movements of Gammarus continue normally in a solution of three chlorides shows plainly that anions of higher valency are of less significance in this process than are the metallic ions.

4. The conception of physiologically balanced salt solutions presupposes that in such a solution the various ions must be present in a definite ratio. If such a solution becomes poisonous when one constituent is removed from the solution it is still not proven that this constituent in itself is necessary for the life of the animal; but there exists the possibility that this constituent is necessary only to counteract the toxic effect of one or more of the other constituents of the solution. I have furnished the proof for this in the case of the egg of Fundulus. This egg develops just as well in distilled-water as in sea-water and hence needs none of the constituents of the sea-water for its development. When a newly fertilized egg is placed in a pure sodium chloride solution of the concentration which this salt has in sea-water it dies in a relatively short time. If, however, a bivalent metallic ion be added, for example, Ca, Mg, Ba or even Pb or Zn, in a very small concentration, the eggs develop.[2] The Ca in the sea water is thus only necessary to prevent the poisonous action of the sodium chloride in the sea-water (either the Na or Cl ions). $ZnSO_4$ by itself is poisonous in the concentration in which it must be used in order to remove the toxic effect of the sodium chloride solution; together with the latter it is harmless; hence the sodium chloride also removes the poisonous effect of the $ZnSO_4$ solution.

Similar relations exist for Gammarus. I have mentioned above that in a pure $\frac{3}{8}$ m sodium chloride solution the respiratory movements cease about as quickly as in distilled water; and that the animals die in a solution of the remaining constituents of the sea-water, more quickly than in distilled water. From this I concluded that sodium chloride on the one hand removed the

[1] Loeb; Archiv für die ges Physiologie, Bd. 91, s. 248. 1902.

[2] Loeb; American Journal of Physiology. Vol. 6, p. 411. 1901.
Loeb and Gies; Archiv für die ges Physiologie, Bd. 93, S. 246. 1902.

poisonous effect of all or some of the other constituents of the sea-water, and vice versa. The correctness of this idea is proven still further in a very striking way by the following experiment. I had already shown in the case of Fundulus eggs that if the concentration of the sodium chloride solution is increased the concentration of the antitoxic bivalent kation must also be increased. This may be shown also for Gammarus. If a $\frac{3}{8}$ m solution of 100 NaCl, 2.2 KCl, 1 $CaCl_2$ be prepared and this solution be diluted by the addition of distilled water or a $\frac{3}{8}$ m sugar solution, the curve of the duration of life, as a function of the concentration, corresponds almost exactly with the corresponding curve in the dilution of sea-water. In a mixture of 10 ccm. of the $\frac{3}{8}$ m solution of 100 NaCl 2.2 KCl, 1 $CaCl_2$ and 90 parts of distilled water Gammarus lives at room temperature five hours or longer. In this solution the Na, K, and Ca salts are diluted to the same degree. If, however, the concentration of the sodium chloride alone be decreased while both the other salts are present in the concentration in which they are contained in normal sea-water the animals die even more quickly than in distilled water. The data of Table II illustrate this.

TABLE II.

	Nature of the solution.	Duration of life of the animals (Gammarus).
1.	10 ccm. $\frac{3}{8}$ m NaCl + 90 ccm. H_2O	40 minutes.
2.	10 " $\frac{3}{8}$ m NaCl + 0.2 ccm. $\frac{3}{8}$ m KCl + 0.1 ccm. $\frac{3}{8}$ m $CaCl_2$ + 90 ccm. H_2O	5 hours.
3.	10 ccm. $\frac{3}{8}$ m NaCl + 2.0 ccm. $\frac{3}{8}$ m KCl + 1.0 ccm. $\frac{3}{8}$ m $CaCl_2$ + 90 ccm. H_2O	15 minutes.
4.	100 ccm. $\frac{3}{8}$ m NaCl	25 "
5.	100 " H_2O	25 "

I believe that from this experiment there can be no doubt that in ordinary sea-water the sodium chloride prevents the poisonous action of the potassium and calcium ions contained in the sea-water just as much as that the reverse takes place. This may be especially recognized if the sodium chloride be replaced

by another chloride, or by another substance, *e.g.*, sugar, (see Table III).

TABLE III.

	Nature of the solution.	Duration of life of the crustacia.
1.	10 ccm. $\frac{3}{8}$ m cane sugar + 90 ccm. H_2O	30 minutes.
2.	10 " " " " + 0.2 " $\frac{3}{8}$ m KCl + 0.1 ccm. $\frac{3}{8}$ m $CaCl_2$ + 90 ccm. H_2O	12 "
3.	10 ccm. $\frac{3}{8}$ m cane sugar + 2.0 ccm. $\frac{3}{8}$ m KCl + 1.0 ccm. $\frac{3}{8}$ m $CaCl_2$ + 90 ccm. H_2O	8 "

In the experiments mentioned in Table III in which solutions without sodium chloride were used, the addition of $CaCl_2$ and KCl increased the toxicity of the solution; and it made little difference whether the two salts were added in the concentration which they possess in mormal sea-water or in a ten-fold dilution.

On the other hand it is shown that if a $\frac{3}{8}$ m NaCl solution be used the Ca and K salts must be added in approximately the concentration in which they exist in sea-water, or perhaps more correctly, it would not do to add them in ten fold dilution. Within certain limits variations in the proportion of these salts are possible but the best results are apparently produced if the concentration of sodium chloride and the other salts are changed in the same proportion. With very low concentrations this may perhaps be different. I will not go into all the details of these experiments since I have already thoroughly dealt with the conception of physiologically balanced salt solutions in earlier papers.[1]

[1] These investigations have remained unknown to Overton as also those investigations in my laboratory which had to do with the relation of sodium ions to muscular activity. Herbst also seems not to have followed these researches since he ascribes to Howell ideas which originated with me and which are in opposition with Howell's ideas. Howell assumed, for example, that the sodium chloride in the blood served only to keep up the osmotic pressure in the tissues but was itself indifferent, and that the calcium alone formed the stimulus for the heart contraction. I showed on the contrary, that the rhythmical contractions depend on the value of the quotient of the concentration of the sodium ions divided by the concentration of the calcium ions and that within certain limits an increase in the concentration of the sodium ions causes an increase in the rate of contraction while an increase in the concentration of calcium ions has the opposite effect.

All these facts seem to show that the rapid death of Gammarus in distilled water depends on the diffusion of Na, Ca, K and possibly Mg salts or ions from the animal. If this is correct the diffusion of electrolytes from the tissues of the animal must occur rather rapidly.

5. As far as my experience goes we must divide marine animals into three groups according to their behavior towards distilled water. The first group consists of forms for which distilled water is entirely harmless. To this group animals such as Fundulus belong. The eggs of this animal do not swell if they are brought suddenly from sea-water into distilled water and they do not shrink with the reverse change of conditions. One might be inclined to assume that these animals are surrounded by an absolutely impermeable membrane. This would be an error, since, in the first place, oxygen and carbonic acid pass, in a state of solution, through the membranes of the animals or of their eggs, and since, moreover, salts and ions pass through the membranes of the animals in both directions. A relatively small increase of the concentration of potassium salts of the sea-water, for example, causes the heart in embryos to cease to beat, and if the egg be brought soon enough into a medium with less potassium the heart again begins to beat. It might be thought from this that the animal is permeable for ions but not for water. Then, however, the question remains unanswered as to why the animal does not become impoverished in salts in distilled water and so die. I believe that we must acknowledge that besides differences of osmotic pressure there must be taken into account other conditions for the exchange of fluids in animals like Fundulus. The secretion of the kidneys and the absorption of fluid in the intestines show that for the explanation of the movements of fluids in the living organs differences of osmotic pressure alone are not sufficient. For animals like Fundulus a solution of Mg isosmotic in the sea-water is much more deleterious than distilled water.

The second group includes those animals or tissues which behave as if they were surrounded by a semi-permeable wall which is easily permeable for water, but impermeable or less permeable for all or many salts. The red blood corpuscles

represent this type. The eggs of the sea-urchin also behave to a certain extent in this way. If these eggs are placed in distilled water they swell up and shrink again if they are returned from the distilled water to the sea-water. In these forms distilled water has a mechanical action. This mechanical action may in itself inhibit protoplasmic movement. If sea-urchin eggs be placed in very dilute sea-water the processes of cell division cease. These processes, however, go on again if the eggs are returned soon enough to the normal sea-water. In distilled water a muscle swells up so that its power of contraction ceases; while this power returns if the muscle be removed from the water quickly enough. Since this mechanical swelling does not take place in isotonic sugar solutions, in the case of the animals and tissues of this group, a dilution of the sea-water or serum with isotonic sugar solutions is more harmless than a dilution with distilled water. An isotonic sugar solution is, however, also poisonous for these animals as is proved by my earlier experiments and as I will show in a later investigation. This toxicity depends, at least in part, on the fact that salts or ions diffuse from the animal or the tissues into the surrounding medium. I suspect that with regard to the permeability of semi-permeable membranes for salts and ions, great differences exist in the different representatives of this group. The living muscle is certainly relatively easily permeable to potassium, sodium, and calcium salts as the observations on the influence of these salts on rhythmical contractions show satisfactorily.

As an example of the third group we may consider Gammarus. For this animal a sugar solution isotonic with sea water is as poisonous as distilled water. In this case the toxicity of both media depends apparently on the diffusion of salts or ions from the tissues.

All three groups have this characteristic in common, namely, that salt solutions which are isotonic but not physiologically balanced are poisonous for them, and in the case of marine animals (such as Fundulus) often more poisonous than distilled water.

6. The results which are contained in the foregoing experiments are as follows:

(*a*) For the kind of Gammarus used in these experiments distilled water and a sugar solution which is isosmotic with sea-water are almost equally poisonous. In both solutions the animals die at room temperature in about half an hour. Hypotonic sugar solutions are not quite so poisonous as sugar solutions which are isosmotic with sea water. The difference is, however, small.

(*b*) If sea water be diluted by the addition of distilled water, the duration of life decreases at first only slightly with an increase in the degree of dilution. But as soon as a dilution of ten times is reached an abrupt decrease in the duration of life takes place with further dilution. Whether the curve of the duration of life at this place is discontinuous is not yet proven. If the sea-water be diluted with an isotonic or weaker sugar solution the curve of the duration of life deviates little from that with dilution by means of distilled water.

(*c*) In a pure sodium chloride solution, isosmotic with sea water or less concentrated, the respiratory movements of Gammarus cease about as quickly as in distilled water.

(*d*) In a solution which contains all other constituents of the sea-water but no sodium chloride, the animals die still more quickly than in distilled water.

(*e*) If a solution be employed which contains NaCl, KCl, and $CaCl_2$ in the concentration in which the salts of Na, Ca, and K are contained in sea-water, Gammarus lives in such a solution for many days. The addition of $MgCl_2$ lengthens the duration of life still more; the addition of other constituents of the sea-water seems, however, to have no further power of prolonging the life of the animals.

(*f*) It follows from these facts that in the first place the sodium chloride contained in the sea-water is poisonous when it alone is present; and Gammarus dies quickly in such a solution. The toxicity of the NaCl is prevented by the Ca and K salts contained in the sea-water. When present alone the K and Ca ions contained in the sea-water are also and to a still greater extent poisonous. Their toxic effect is counteracted by the sodium chloride contained in the sea-water.

(*g*) For the mutual counteraction of these poisonous effects

it is necessary that the concentration of the salts or ions which act antagonistically, should stand in a definite relation to one another. This relation represents approximately that in which they are in normal sea-water. If one takes a solution in which the sodium chloride is diluted tenfold, while K and Ca are present in the concentration in which they exist in normal sea water, the animals die in such a solution in about a quarter of an hour. If, however, a solution of the same salts be used in which not only the sodium chloride, but also the K and Ca salts are diluted tenfold the animals may live five hours. These facts support the theory already advanced by me at an earlier date that the sea-water is a physiologically balanced salt solution for the animals contained in it and that the serum of an animal is a similar solution in relation to the tissues of the animal. It is a solution in which there is a mutual prevention of the poisonous actions, which the individual constituents have if they are alone in solution. If the changes of the concentration of the individual constituents of such a physiologically balanced solution take place within certain narrow limits one can call forth in the tissues properties which they do not normally possess, such as the artificial parthenogenesis, rhythmical twitchings, and the increase in the irritability of the muscles and nerves. I am of the opinion that this point of view will prove fruitful in pathology (especially in the study of nervous diseases).

(*h*) The experiments give grounds for the idea that the toxic action of distilled water and the sugar solution on Gammarus depends essentially on the fact that electrolytes or ions leave the body. Possibly the exit of antagonistic salts or ions takes place with unequal rapidity or in unequal relations. Such a disturbance of the relations of the concentration of antagonistic salts or ions in the tissues of the animals must hasten the onset of death.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 8, pp. 71–75 November 30, 1903

ON THE SEGMENTAL CHARACTER OF THE RESPIRATORY CENTER IN THE MEDULLA OBLONGATA OF MAMMALS.[1]

BY

JACQUES LOEB.

1. The researches of Grünbaum and Sherrington[2] have established a regular arrangement of the points of stimulation in the cerebral cortex of the ape which corresponds to a large extent with the arrangement of the segments in the spinal cord. This fact supports the segmental theory of the functions of the nervous system, that is, the idea that in higher animals, as in the lower, the central nervous system is essentially only a series of segmental ganglia. It is known that this theory, which is based upon comparative physiology, is opposed by another theory, namely, the center theory, according to which complicated functions of the whole body are localized in a limited area of the brain or of the medulla oblongata. I have mentioned the reasons which speak in favor of the segmental theory in the higher animals in the German and still more completely in the English edition of my "Comparative Physiology of the Brain." Since recently the papers of Babak[3] and Philipson[4] show that the interest in the segmental conception of the central nervous system is increasing

[1] Translated from Pflüger's Archiv, vol. 96, p. 536. 1993, by J. B. MacCallum.

[2] Grünbaum and Sherrington; Proceedings of the Royal Society, vol. 69, p. 206. 1901.

[3] Babak; Archiv für die ges. Physiologie. Bd. 93, S. 134. 1902.

[4] Philipson; Comptes rendus de l'académie des sciences, vol. 136, p. 61. 1903.

I wish to point out that a well-known but apparently contradictory fact harmonizes with this theory, namely, the existence of a respiratory center in the medulla oblongata of mammals. The essential respiratory muscles in these forms are as is well-known, the costal muscles and the diaphragm, the segmental ganglia of which all lie in the cervical and dorsal regions of the cord. Nevertheless, all movements of respiration cease if a section be made through the spinal cord between the medulla oblongata and the origin of the phrenic. From the standpoint of the segmental theory two things remain unexplained; in the first place how it happens that a respiratory centre exists in the medulla oblongata without the presence of a peripheral respiratory organ corresponding to these segments; secondly, how it happens that after the destruction of this respiratory center, *e.g.*, after the separation of the spinal cord from it, the automatic activity of the segmental ganglia lying below the section ceases in mammals. It is upon this fact that the center theory bases its idea that all functions are centralized in the brain or the medulla oblongata. That in lower animals the facts directly contradict this idea is explained by the assumption that in lower animals the segmental theory may be correct but in higher animals "the functions have migrated to higher levels." One may seek to form, however, a definite idea of the wandering of functions in the central nervous system and one will find that this form of expression can only conceal our ignorance of the actual relations, but cannot remove it.

2. I have pointed out in my "Comparative Brain Physiology" that we must go back to the embryonic conditions in higher animals if we wish to arrive at the proper idea of the relations of the central nervous system to the peripheral organs. The reason for this lies in the fact that the peripheral organs, during embryonic development undergo different and, as a rule, much more extensive shiftings and transformations than the corresponding parts of the spinal cord. If we consider, however, the early embryonic conditions we notice that in spite of the change and the shifting of the peripheral organs during development the segmental character of the innervation is preserved. As a striking example of this I mentioned

the relation of the phrenic nerve to the diaphragm. The phrenic originates in the cervical cord; and before the embryology of the diaphragm was known one might have imagined that here also was a contradiction of the segmental theory, since the diaghragm which forms the floor of the thorax was innervated by a center lying high up in the cervical cord. The embryological observations, however, have shown that the diaphragm is formed in the same segments of the body of the embryo in which the phrenic arises, and that only secondary embryonic processes of growth push the diaphragm from its place of origin towards the floor of the thorax. Only the disregard or ignorance of embryological facts could make the supposition possible that the innervation of the diaphragm is an exception to the segmental theory.

Exactly in the same way, according to my opinion, does the matter stand in reference to the respiratory center of the medulla oblongata of mammals. In adult mammals no peripheral segmental respiratory organ corresponds with this "center." If we turn, on the contrary, to the embryo we find, I believe, such a structure. *I regard it as possible that the respiratory center of the medulla oblongata contains the segmental ganglion or the segmental ganglia for a peripheral respiratory organ which exists only temporarily in the embryonic life of man, namely, the gills.*

If this is correct it is to be expected that the nerves for the gills, or more correctly the respiratory muscles, in fishes belong to the vagus group, which is actually the case. Especially it follows from the work of C. J. Herrick[1] that the respiratory ganglia in the medulla oblongata of mammals are entirely homologous, with the segmental ganglia for the innervation of the gills of fishes in the lobus vagi.[2]

The essential difference between the respiratory ganglia in the medulla oblongata in fishes and in mammals is the lack of the tract of fibres to the nucleus of the phrenic in fishes and consequently the lack of the so-called noeud vital.[3] Since in fishes breathing is limited to the gills and no respiratory organs

[1] C. J. Herrick; The Journal of Comparative Neurology. Vol. IX, p. 153. 1899.
[2] Dr. Hardesty at my suggestion has been so kind as to verify the last point.
[3] The term noeud vital should be eliminated from physiology.

exist in the deeper lying segments there is naturally no so-called noeud vital possible in these animals. The discovery of embryonic gills by Rathke, a discovery which has justly excited much admiration furnishes also support for the segmental theory of the respiratory center in the medulla oblongata of mammals.

3. The "respiratory center" in the medulla oblongata is, if we consider the embryological facts, and this is essential in brain physiology, not a ruling center but a segmental ganglion, or rather a series of segmental ganglia. Then one difficulty still remains, namely, why it is that the nucleus of the phrenic and the ganglia of the costal respiratory muscles are no longer automatically active when they are separated from the respiratory ganglia (the gill ganglia) of the medulla oblongata, or when the latter ganglia are destroyed.

I am disposed to believe that in this field discoveries are still to be made, important theoretically and for practical purposes, in which it will be possible to show that the relation of the different respiratory ganglia is similar to that of the different parts of the heart. While the auricle of the heart of the tortoise if it be separated from the rest of the heart continues to contract in the original rhythm, the separated ventricle comes to a standstill. This does not show that the ventricle is not capable of activity but only that the physico-chemical conditions for the automatic activity of the ventricle are somewhat different from those for the auricle. By a series of researches in my laboratory the idea has been arrived at that the irritability of the nerves and muscles and the rhythmical activity of different organs is, amongst others, a function of the quotient $\frac{C_{Na}}{C_{Ca}}$, that is, the concentration of the sodium ions divided by the concentration of the calcium ions of the solution surrounding the tissues in question. In the blood of the tortoise the value of this quotient is great enough to allow the rhythmical contractions of the isolated auricle, but too small to permit of rhythmical contractions of the isolated ventricle.[1] A similar condition is present in the rhythmical contractions of a medusa (Gonionemus). If the margin be separated from the center of this medusa only the

[1] The latter contracts in the body rhythmically only so long as it is in connection with the auricle and receives its impulse from this part of the heart.

former is in a position to beat in sea-water. If the value of the quotient $\frac{C_{Na}}{C_{Ca}}$ be raised in sea water by a lessening of the concentration of the calcium ions or by an increase in the number of sodium ions rhythmical contractions of the center set in.

I regard it as possible that something similar is true for the segmental respiratory ganglia. It may be that something in the composition of the blood allows the automatic activity of the uppermost segmental respiratory ganglia (the gill ganglia) to go on while this is not the case for the cervical and thoracic respiratory ganglia.[1]

[1] I have already mentioned in my Comparative Brain Physiology that the physico-chemical conditions for the activity of the head and body segments are in general somewhat different, as is the case also for the center and the border of Gonionemus.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 9, pp. 77–79 December 24, 1903

ON THE PRODUCTION AND SUPPRESSION OF GLYCOSURIA IN RABBITS THROUGH ELECTROLYTES.

(A Preliminary Communication.)

BY

MARTIN H. FISCHER.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

I.—Introduction.

In 1871 Bock and Hoffman described as a new method of producing glycosuria the infusion of large amounts (20–25 c.c. or more during each 5 minutes) of a one per cent sodium chloride solution into the circulation of rabbits. If the experiment is carefully performed the infusion is first followed by an increased diuresis and some time later sugar appears in the urine. Külz seems to have been equally successful in bringing about glycosuria by this means, as also by the injection of solutions of sodium acetate, sodium carbonate, sodium valerianate and sodium succinate into the circulation.

The introduction of large amounts of these salt solutions into the circulation brings about two changes,—an increase in the amount of water and an increase in the amount of sodium salts contained in the animal.

Loeb's investigations have shown that by an increase in the concentration of sodium ions the irritability of nerves and muscles is increased and rhythmical contractions may be produced; and that the addition of calcium salts counteracts these effects. Dr. Loeb pointed out to me that the glycosuria in rabbits after the

injection of solutions of sodium salts was probably an analogous case with the production of muscular twitchings by the same salts, and that it was therefore to be expected that two series of phenomena might be discovered in regard to glycosuria,—first, that those salts which are especially powerful in the production of hypersensitiveness and twitchings (*e.g.*, the citrates) should also be especially powerful in the production of glycosuria; and second, that calcium salts should counteract the glycosuria produced by the sodium salts. At Dr. Loeb's suggestion I undertook a series of experiments to test this view.

II.—Experimental Results.

1. The injection of a $\frac{1}{6}$ molecular NaCl, NaBr, or NaI solution into the circulation of rabbits brings about a polyuria and usually a glycosuria. The polyuria evidences itself in ten to fifteen minutes after the beginning of the injection and lasts as long as the injection is continued (27 hours). The glycosuria, when it appears at all, does not evidence itself until one to four hours after the beginning of the injection and lasts six to eight hours. The amount of sugar excreted in the urine is at first small, rapidly increases to a maximum, and then gradually falls to zero. The amount of sugar excreted when the excretion is at its height, lies above 0.2%. I shall give more accurate figures in a later publication. When the excretion of sugar has fallen to zero continued infusion of the sodium salts causes no more sugar to appear in the urine, even though the polyuria continues as before.

2. If a less concentrated (for example, a $\frac{1}{7}$ molecular) solution of NaCl than a $\frac{1}{6}$ molecular is injected intravenously, polyuria is still brought about but no glycosuria.

3. Glycosuria does not follow the injection of a mixture of $\frac{1}{6}$ molecular NaCl solution with $CaCl_2$. Polyuria, however, appears in the same way as when NaCl is given alone.

4. If glycosuria is started in an animal by the intravenous injection of pure solutions of NaCl, NaBr or NaI, the excretion of sugar can be markedly lessened or suppressed entirely by substituting for the $\frac{1}{6}$ molecular NaCl, NaBr, or NaI solutions a

mixture of one of these with $CaCl_2$ (for example, 975 c.c. $\frac{1}{6}$ m NaCl + 25 c.c. $\frac{3}{8}$ m $CaCl_2$).

5. Animals which will excrete no sugar in their urine when $\frac{1}{6}$ molecular NaCl alone is injected can usually be caused to excrete sugar by substituting for the pure NaCl solution a mixture of $\frac{1}{6}$ molecular NaCl with sodium citrate. When sodium citrate is given to animals which are already excreting a small amount of sugar the excretion of sugar is increased.

6. The amount of urine and the amount of sugar excreted bear no relation to each other and vary independently of each other.

7. Albuminuria is a frequent though not constant accompaniment of glycosuria.

8. The animals seem to vary in regard to the ease with which glycosuria can be produced in them. Occasionally one finds rabbits which are so sensitive to pure NaCl solutions that they excrete traces of sugar after the injection of $\frac{1}{7}$ molecular NaCl. In those which excrete no sugar after prolonged injection with $\frac{1}{6}$ molecular NaCl, glycosuria has thus far always been produced when a small amount of sodium citrate has been added to the NaCl solution. Some difference seems to exist also, in regard to the ease with which the excretion of sugar can be suppressed in different animals. Some animals cease excreting sugar sooner and after smaller doses of $CaCl_2$ than others.

9. A considerable latent period exists before the specific effects of the various salts show themselves. When the excretion of sugar has been started by a pure NaCl solution, for example, it does not cease, for thirty to sixty minutes after the injection of 975 c.c. $\frac{1}{6}$ m NaCl + 25 c.c. $\frac{3}{8}$ m $CaCl_2$ is begun. After the excretion of sugar has once been stopped by this means it cannot be started again for an hour or two after the return to the pure NaCl solution.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 10, pp. 81–82 January 15, 1904

THE INFLUENCE OF CALCIUM AND BARIUM ON THE FLOW OF URINE.

(A Preliminary Communication.)

BY

JOHN BRUCE MacCALLUM.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

1. It is well known that the flow of urine may be considerably increased by the administration of certain salts—the so-called saline diuretics.

2. I have found in dogs and rabbits that the quantity of urine may be markedly diminished and in some cases, for a time, almost entirely inhibited by the introduction of $CaCl_2$ into the circulation.

3. Calcium chloride diminishes not only the normal flow of urine, but also that which is caused by the administration of the saline diuretics. For example, the rate of secretion which has been largely increased by the intravenous injection of normal salt solution may be temporarily lessened to a marked extent by the introduction of $CaCl_2$ into the blood.

4. In all cases $\frac{m}{8}$ solutions were used, and $\frac{m}{8}$ NaCl solution was introduced into the blood at a constant rate throughout the experiments. After a short time the rate of secretion became constant. It was then found that the addition of a small quantity of $BaCl_2$ ($\frac{1}{8}$ cc. $\frac{m}{8}$ solution) to the blood causes a marked increase in the flow of urine so that the amount of fluid excreted may considerably exceed that which is introduced into the blood during the same period of time.

5. This action of barium is counteracted by the injection of $CaCl_2$.

6. If a larger quantity of $BaCl_2$ (1 cc. $\frac{m}{8}$ solution) be added to the blood the flow of urine ceases abruptly and complete anuria ensues. In some cases the injection of $CaCl_2$ abolishes this inhibitory action so that the urine flows again. Usually however this action of barium persists.

7. The fact that barium when given in smaller and in larger doses may thus apparently have opposite effects on the flow of urine may possibly be explained by analogy with the action of barium on the intestine. I have already shown that barium chloride causes not only an increase in the secretion of fluid into the intestine but also active peristaltic movements, and violent local constrictions of the intestine. Similarly very small doses of $BaCl_2$ increase the secretory activity of the kidney. It seems probable however that the cessation of the flow of urine which follows the injection of larger quantities of the salt is due not to an inhibition of secretion, but to the action of the barium on the muscular coats of the urinary passages, especially of the calyces and pelvis of the kidney, and of the ureter. This action would bring about a constriction of the tubes and a closure of the lumen. The fact that calcium counteracts both effects of the barium supports this explanation.

8. The actions of calcium and barium on the flow of urine are in every way analogous to their influence on the intestine which I have previously described. The suppression of the urinary secretion by calcium also is analogous to the suppression of twitchings in voluntary muscle by calcium which has been described by Loeb.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 11, pp. 83–85 February 10, 1904

FURTHER EXPERIMENTS ON THE FERTILIZATION OF THE EGG OF THE SEA-URCHIN WITH SPERM OF VARIOUS SPECIES OF STARFISH AND A HOLOTHURIAN.

BY

JACQUES LOEB.

1. A continuation of my experiments on the fertilization of the eggs of the sea-urchin *(Strongylocentrotus purpuratus)* with the sperm of starfish has yielded the result, that these eggs can be fertilized and caused to develop not only with the sperm of Asterias but also with the sperm of a brittle-star and the sperm of the twenty-ray starfish *(Pycnopodia spuria)*. I have furthermore succeeded in fertilizing the same eggs and in causing them to develop with the sperm of a Holothurian *(Cucumaria)*.

2. The artificial solutions in which the egg of the sea-urchin can be fertilized with the sperm of the brittle-star and *Pycnopodia* were found to be identical with those in which the same egg can be fertilized with the sperm of *Asterias*.

3. In my previous communications I have mentioned the fact that in the running seawater of the laboratory the egg of *Strongylocentrotus* could not be fertilized with the sperm of *Asterias*; and with sea-water taken directly from the ocean the hybridization was sometimes possible, sometimes impossible. The same statement holds good for the fertilization of the same egg with the sperm of the brittle-star. As far as the fertiliza-

tion of sea-urchin egg with the sperm of *Pycnopodia* is concerned I have not yet seen it succeed in normal seawater, though further experiments may give better results.

4. In a former publication I have expressed the belief that the addition of a slight amount of Na_2CO_3 to sea-water might render the latter almost as favorable for these hybridizations as the optimal artificial solutions used in my former experiments. This idea was tested and found to be correct for the hybridization of the sea-urchin egg with the sperm of both kinds of starfish. The addition of about 0.4 cc. of a $\frac{m}{8}$ solution of Na_2CO_3 to 100 cc. of seawater sufficed to accomplish this result.

5. The addition of the same amount of carbonate to sea-water made the fertilization of the egg of the sea-urchin with the sperm of its own species difficult or practically impossible, provided the eggs and sperm were washed for three or four minutes in this alkaline sea-water before they were mixed. This fact is in harmony with the fact previously found that the artificial solutions which are optimal for the hybridization of the sea-urchin egg with the starfish sperm make the fertilization of the same eggs with sperm of their own kind difficult if not impossible.

6. There was always a typical difference between the hybridization in sea-water to which sodium carbonate had been added and in normal sea-water. If in the latter the eggs of the sea-urchin could be fertilized at all with the sperm of the brittle-star the fertilization occurred as a rule only many hours (six to ten or more) after the addition of the sperm, while in the sea-water to which sodium carbonate had been added or in the optimal artificial solutions the eggs of the same female are fertilized by the sperm of the same male in less than half an hour.

7. This fact and the fact that the possibility of fertilization depends on the presence or absence in the surrounding solution of mere traces or very minute quantities of certain substances (*e.g.* hydroxylions) give rise to the idea that the main variable determining the entrance of the spermatozoon into the egg may be of the nature of surface tension or a function of the latter. This idea would also harmonize with the fact that there are great individual variations in the sperm of starfish of the same species in its power of fertilizing the eggs of the sea-urchin.

8. It has been mentioned in my previous papers that these facts may have some bearing on the problem of infection and immunity. Especially that which we call the predisposition for an infection may depend on equally slight variations in the constitution of the liquids of our body as the variations on which the fertilization of the sea-urchin egg with the sperm of the starfish in sea-water depends. If our supposition be correct that in the latter case the controlling variable is of the nature of surface tension it stands to reason that the variable in the case of individual predisposition for infection is also of this nature.

9. The solutions in which the fertilization of the eggs of *Strongylocentrotus* with the sperm of the *Holothurian* was accomplished were different from those in which the same eggs could be best fertilized with the sperm of starfish. As I have not yet finished my experiments with Holothurian sperm I shall reserve the statement concerning the conditions for the fertilization of the sea-urchin egg with the sperm of *Holothurians* for the main paper.

10. I have not yet made any special effort to raise the hybrids mentioned in this paper but have noticed that their development is decidedly retarded compared with that of the pure larvae of *Strongylocentrotus*.

I am indebted to Professor Heath and Dr. Carlson of Stanford University for their kindness in pointing out to me the collecting ground for some of the material used in these experiments.

The experiments are being continued.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 12, pp. 87–113 February 15, 1904

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ON THE PRODUCTION AND SUPPRESSION OF GLYCOSURIA IN RABBITS THROUGH ELECTROLYTES.

(Second Communication.)[1]

BY

MARTIN H. FISCHER.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

I.—Introduction.

In 1871 Bock and Hoffmann[2] described as a new method of producing glycosuria the infusion of large amounts (25–30 c.c. or more during each 5 minutes) of a one per cent sodium chloride solution into the circulation of rabbits. If the experiment is carefully performed the infusion is first followed by an increased diuresis and some time later sugar appears in the urine. Külz[3] seems to have been equally successful in bringing about glycosuria by this means, as also by the injection of solutions of sodium acetate, sodium carbonate, sodium valerianate, and sodium succinate into the circulation.

The introduction of large amounts of these salt solutions into the circulation brings about two changes,—an increase in the amount of water and an increase in the amount of sodium salts contained in the animal.

[1] The first communication was published December 24, 1903. University of California Publications. Physiology, 1903, I, p. 77.

[2] Bock, C., and Hoffmann, F. A.: Reichert and Du Bois-Reymond's Archiv, 1871, p. 550.

[3] Külz, E.: Eckhard's Beiträge, 1872, vi, p. 117 (Cited from Pflüger, E.: Pflüger's Archiv, 1903, xcvi, p. 313.)

The investigations of Loeb[4] have shown that by a number of sodium salts muscular twitchings can be produced, and that the addition of calcium salts counteracts this effect. Those sodium salts whose anions are liable to diminish the concentration of free calcium ions in the blood or tissues act much more powerfully than do the other sodium salts. For this reason a pure solution of sodium citrate acts more powerfully than an equimolecular solution of sodium chloride, sodium bromide, sodium iodide, or sodium nitrate. These muscular twitchings are done away with when a small amount of any soluble calcium salt is added to the solution of the sodium salt. Dr. Loeb pointed out to me that the glycosuria in rabbits after the injection of sodium salts was probably an analogous case with the production of muscular twitchings by the same salts; and that in consequence two series of phenomena might be discovered in regard to glycosuria,—first, that those salts which are especially powerful in the production of hypersensitiveness and twitchings (*e.g.*, the citrates, oxalates, etc.) should also be especially powerful in the production of glycosuria; and second, that calcium salts should counteract the glycosuria produced by the sodium salts. The following series of experiments was undertaken to test this view.

II.—Methods of Experiment.

General Remarks.—Rabbits were used throughout in the following experiments. In addition to the ordinary diet of barley and alfalfa hay the rabbits were fed carrots. The last named food is, however, not nesessary for the success of the experiments. Strong, healthy rabbits were used throughout, varying in weight from 3 to 4 kilos.

In order to inject the large amounts of sodium chloride solution which are necessary for the production of glycosuria, Bock and Hoffmann inserted canulae into the peripheral end of either the femoral or carotid arteries. As this procedure necessitates considerable injury to the animal, and is one which does not per-

[4] Loeb, J.: Festschrift für Fick. Braunschweig. 1899. American Journal of Physiology, 1901, v. p. 362. Pflüger's Archiv, 1902, xci, p. 248. Decennial Publications, University of Chicago, 1902, x.

mit the experimenter to change rapidly from one blood vessel to another when the canula becomes closed through coagulation of the blood, I substituted for the canula two hypodermic needles connected with the injection apparatus shown in Fig. 1.

Fig. 1.

F is a fresh water tap communicating with the large pressure bottle *A*. When the water is turned on, the air in *A* is compressed and moves over into the graduated cylinders *B* and *C* through the rubber tubes *m* and m_1. The cylinders *B* and *C* are filled with the solutions which are to be injected into the animal. The pressure of the air from *A* on the surface of these solutions causes them to escape at *N* and N_1, after passing through the tubes *g n o* and g_1 n_1 o_1. *N* and N_1 represent two hypodermic needles fastened into the rubber tubes *o* and o_1. *xx* and x_1x_1 indicate the position of four Hoffmann clamps. By closing *x* and *x* while x_1 and x_1 are open, only the solution contained in *B* can pass into the tubes *o* and o_1. By closing x_1 and x_1 while *x* and *x* are open, only the solution contained in *C* can pass into the tubes *o* and o_1. By properly manipulating these clamps one

can therefore at will quickly change from the injection of one solution to the injection of another without disturbing the rest of the apparatus or the animal.

The injections of salt solutions were made into the lateral veins of the ears. When from oedema at the base of the ears, or from some other cause, it was necessary to move the needles to some other portion of the body, they were inserted into one of the superficial branches of the femoral vein which lie on the inside of the thigh near the knee, and which are readily exposed by a small incision through the skin. This method does away with any extensive operating and leaves the rabbits practically unharmed; in fact I have several times been able to use the same animal over again for these experiments on a subsequent day. This method, it seems to me, reduces to a minimum any sources of error which might creep into the experiments.

The urine was obtained by catheterizing the bladder. This is always possible in the males. In those females in which this was impossible, the bladder was emptied by gentle pressure backwards and downwards on the abdominal wall.

To guard against loss of heat the animals were warmly wrapped up in towels. The salt solutions were heated to body temperature before being injected; and to keep them warm the cylinders containing the injection fluids and the rubber tubes carrying the solutions into the animal were kept in hot water.

Determination of Sugar.—A standard Fehling's solution was used to detect the presence of and determine the amount of sugar contained in the urine. Analyses of the urine were made every seven and one-half or fifteen minutes. The method of making these analyses was as follows: In order to precipitate the albumin which appears from time to time, each specimen of urine was rendered faintly acid by the addition of a drop of acetic acid and boiled. After carefully neutralizing the acid and rendering the urine faintly alkaline by the addition of potassium hydroxide, the urine was filtered. To five cubic centimeters of this urine were added varying amounts of freshly prepared Fehling's solution. The mixture was then boiled. A yellow or orange red turbidity or precipitate of cuprous oxide was considered evidence of the presence of a reducing sugar.

The Fehling's solution was not mixed until just before using, nor was it employed before prolonged boiling had shown that it itself contained no reducing substances.

By using measured amounts of urine and measured amounts of Fehling's solution, one can readily detect any increase or decrease in the amount of sugar excreted in the urine. In my first experiments I was content to know whether the amount of sugar was rising or falling. In my later experiments I tried to determine the absolute amounts of sugar. This was possible, however, only in a few instances. Usually I had to content myself, because of the limited time allowed for each analysis, by saying that the amount of sugar lay above or below a certain per cent.

III.—On the Production of Glycosuria by Sodium Chloride.

If 75–100 c.c. of a $\frac{1}{6}$ molecular NaCl solution are injected during each fifteen minutes into the circulation of a large rabbit one usually observes the following phenomena. Within fifteen minutes after the beginning of the injection the *quantity of urine excreted increases*. In an hour the quantity of urine has risen to a point where it parallels roughly the amount of salt solution which is injected into the animal.

The character of the urine is also markedly altered. The urine which was at the beginning of the experiment turbid, deep yellow and usually alkaline to litmus paper becomes clear and pale in color in the course of half an hour. The reaction, too, changes to neutral, or more frequently to faintly acid as indicated by litmus paper. *The excretion of this faintly acid and watery urine usually continues as long as the injection is kept up* (twenty-seven hours in one case). Sometimes a transient or persistent hæmaturia develops. The bleeding is usually caused by some injury to the urethra. This is evidenced by the fact that hæmaturia develops most frequently in male rabbits which have been catheterized. Furthermore, post mortem examination has in nearly all such cases shown the presence of ecchymoses in the course of the lower urinary passages.

Sugar appears in the urine in about two hours after the beginning of the injection. When less than 75–100 c.c. of $\frac{1}{6}$ molecular

NaCl are injected during each fifteen minutes, it usually takes longer than two hours before sugar appears in the urine. Individual variations, however, seem to exist among the animals. Certain rabbits begin to excrete sugar in their urine sooner and after the injection of smaller amounts of sodium chloride solution than do others. *The glycosuria lasts six to eight hours.* The amount of sugar excreted in the urine is at first small, rapidly increases to a maximum, and then gradually falls to zero. The concentration of sugar in the urine when the glycosuria is at its height may lie above 0.25%. When the excretion of sugar has fallen to zero, continued infusion of the sodium chloride solution causes no more sugar to appear in the urine, even though the polyuria continues as before.

The following experiment will serve to illustrate the foregoing. The excretion of sugar was obtained in this animal sooner and after the injection of a smaller amount of sodium chloride solution than is usually the case. The upright dagger (†) indicates that the amount of sugar has risen since the last analysis; the inverted dagger (‡) indicates that the amount of sugar in the urine has fallen since the previous analysis. No dagger means that no change has occurred in the amount of sugar excreted in the urine since the last analysis.

Time is indicated in the first column. The second column shows how much salt solution is injected. The figures indicate the amount of salt solution injected during the past fifteen minutes. The amount of urine excreted is shown in the third column. The figures refer to the amount which has been excreted in the past interval of time (usually the last fifteen minutes).

Experiment 1.

Injection fluid: $\frac{1}{6}$ molecular NaCl.

Large female Belgian Hare. Fed over night on carrots in addition to barley and alfalfa hay. Urine obtained by manual pressure over the bladder.

Time.	Amount of salt solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.			
A.M.					
5:55	...	....	Animal bound to table.		
7:10	....	2	Turbid, yellow, alkaline.		No sugar.
7:15	....	....	*Injection of $\frac{1}{6}$ mol. NaCl begun into right ear.*		
7:30	35	5	Getting clearer, yellow, alk.		No sugar.
7:45	40	12	Getting clearer, yellow, faintly acid.	No alb.	Trace sugar.
8:00	30	10	Clear, light yellow, faintly acid.	No alb.	Sugar†; great increase.
8:10	....	22		No alb.	Much sugar.†
8:22	30	2		No alb.	Much sugar.†
8:30	20	6		No alb.	Much sugar.
8:45	15	15		No alb.	Much sugar.
9:00	30	....		.....	....
9:15	40	17		No alb.	Much sugar.
9:30	85	18		No alb.	Much sugar.
9:45	65	20		No alb.	Much sugar.
10:00	40	18	Clear, pale yellow, faintly acid.	No alb.	Much sugar.
10:15	15	30		No alb.	Much sugar.
10:30	25	30		No alb.	Much sugar.
10:45	25	35		No alb.	Much sugar.
11:00		30		No alb.	Much sugar.
11:15	?	7		No alb.	Sugar.↓
11:30	20?	....		.....	....
11:45	....	5		No alb.	Sugar.↓
12:00	20	7		No alb.	Sugar.
P.M.					
12:15	30	20	Slightly turbid, pale yellow.	Trace alb.	Sugar.↓
12:30	30	27		Trace alb.	Sugar.↓
12:45	20	35		Trace alb.	Sugar.↓
1:00	40	30		No alb.	Small amount sugar.↓
1:15	45	33		No alb.	Trace sugar.
1:30	40	27	Clear like H_2O.	No alb.	Trace sugar.
1:45	35	33		No alb.	No sugar.
2:00	40	45		No alb.	No sugar.
2:15	20	55		No alb.	No sugar.
2:30	30?	37		No alb.	No sugar.
2:45	35	13		No alb.	No sugar.
3:00	40	33	Clear like H_2O, acid.	No alb.	No sugar.
3:15	30	52		No alb.	No sugar.
3:30	25	40		No alb.	No sugar.
3:45	40	76		No alb.	No sugar.

Experiment 1.—(Continued.)

Time.	Amount of salt solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.			
P.M.					
4:00	40	50	Clear like H_2O, neutral.	No alb.	No sugar.
4:15	35	32		No alb.	No sugar.
4:30	45	56		No alb.	No sugar.
4:45	30	40		No alb.	No sugar.
5:00	....	....	Injection stopped. Animal loosened from table. The animal is stiff and has a slight oedema of left fore leg around which the cord was tied too tightly. Otherwise the rabbit is well. Returned to hutch.		
	1185	1225			
	Total amount injected	Total amount urine			

Following day, 6:00 A.M. Rabbit is in good condition. Takes food. 6 c.c. of turbid, yellow urine expressed from bladder shows no sugar and no albumin.

The animal remained well and was used for another experiment five days later.

In his observations on muscular twitchings Loeb[5] found that the rapidity with which the contractions appear in pure sodium chloride solutions is dependent upon the concentration of the salt solution in which the muscles are immersed. The muscular twitchings appear the more rapidly (within certain limits) the greater the concentration of the sodium chloride solution.

I tried to see whether a similar relation exists between the rapidity of the appearance of glycosuria and the concentration of the sodium chloride solution injected into the rabbit. If it is true that the glycosuria produced by the injection of a pure sodium chloride solution is due to an increase in the amount of the sodium salts contained in the animal, then strong solutions of sodium chloride ought to bring about glycosuria more rapidly than weaker ones. To test this idea experiments were performed

[5] Loeb, J.: Festschrift für Fick. Braunschweig, 1899.

on eight rabbits of approximately the same weight, chosen from two litters and fed for several days previously on the same kind of food. The same quantity but different concentrations of sodium chloride solution were injected intravenously into these rabbits during each unit of time. *These experiments showed that glycosuria appears the sooner the greater the concentration of the sodium chloride solution which is injected.*

Four experiments were made with $\frac{1}{7}$ molecular NaCl. In three of these experiments no sugar appeared in the urine even though the intravenous injection of the salt solution was kept up seven hours. In the fourth of these experiments a trace of sugar appeared after the injection of $\frac{1}{7}$ molecular NaCl had lasted four hours. This trace disappeared immediately, however, and even though I continued the injection another two hours sugar did not reappear in the urine.

The intravenous injection of a $\frac{2}{13}$ molecular NaCl solution brought about glucosuria in two hours and ten minutes. Only a trace of sugar appeared in the urine, however, and this disappeared after an hour and a quarter, even though the injection of the sodium chloride solution was continued.

A $\frac{1}{8}$ molecular NaCl solution brought about glycosuria in one hour and forty-five minutes. The trace of sugar which appeared in the urine at this time increased in the course of fifty minutes to 0.15%. After the excretion of sugar had continued at this rate for two hours the animal was released from the table.

A $\frac{2}{11}$ molecular NaCl solution brought about glycosuria in sixty-seven minutes. Twenty-two minutes later the concentration of the sugar in the urine was 0.12%.

A $\frac{1}{5}$ molecular NaCl solution brought about glycosuria in twenty-two minutes. Thirty minutes later analysis showed that the urine contained 0.11% and an hour later 0.21% sugar. When this excretion of sugar had lasted two hours longer the animal was released from the table.

The fact that the amount of sugar excreted in the urine rarely exceeds 0.25% does not at first sight give the impression of a severe glycosuria. The gravity of the glycosuria at once evidences itself, however, when it is remembered that the injection of large quantities of water into the animal increases enormously

the amount of urine excreted in the unit of time. In the experiments with $\frac{1}{6}$ molecular NaCl solution, for example, the rabbits excreted in one hour approximately as much as they do normally in twenty-four. This means that were the urine not diluted by the intravenous injection of large amounts of water, the amount of sugar in the urine would at times exceed 6%—a very severe diabetes indeed.

I have begun a series of experiments with the injection of highly concentrated solutions of NaCl. In one such experiment glycosuria followed immediately after the injection of a few cubic centimetres of $\frac{1}{2}$ molecular NaCl. The amount of urine excreted, which was proportionately small, showed the presence of 1.4% sugar at one time.

IV.—On the Production of Glycosuria by Sodium Salts other than Sodium Chloride.

The next question which presented itself was whether the power of NaCl to produce glycosuria was a characteristic of all sodium salts. The fact that Külz[6] had succeeded in bringing about glycosuria by the injection of sodium acetate, sodium carbonate, sodium valerianate and sodium succinate into the circulation pointed in this direction, but that he had been unsuccessful when he employed sodium bromide and sodium iodide solutions spoke against this idea. As Külz had employed only solutions of equal percentage (using, for example, 1% solutions of sodium bromide and sodium iodide) instead of using equimolecular solutions, it seemed probable that his failure to succeed with the last-named salts was due to the fact that his solutions were not concentrated enough. I decided, therefore, to repeat Experiment 1 and substitute for the $\frac{1}{6}$ molecular NaCl solution an equimolecular NaBr or NaI solution, I experimented also with a $\frac{1}{6}$ molecular $NaNO_3$ solution. *Glycosuria followed the intravenous injection of solutions of these salts just as quickly, and in the same way as the intravenous injection of NaCl.* The following experiments illustrate this fact. In order to be brief only the first few hours of the experiments are given in detail.

[6] Loc. cit.

Experiment 2.

Injection fluid: $\frac{1}{6}$ molecular NaBr.

Large male Belgian Hare. Fed on carrots for several days past in addition to barley and alfalfa hay. Urine obtained by catheterization.

Time.	Amount of salt solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.			
P.M.					
4:13	...	....	Animal tied to table.		
4:15	....	4	Slightly cloudy, yellow, alk.		No sugar.
4:25	....	....	*Injection of $\frac{1}{6}$ mol. NaBr begun into superficial branch of right femoral vein.*		
4:30	15	2	Slightly turbid, yellow, alk.		No sugar.
4:45	40	5	Slightly turbid, yellow, alb.		No sugar.
5:00	40	8	Slightly turbid, pale yellow, slightly acid, albumin.		No sugar.
5:15	40	10	Clear, pale yellow, slightly acid.		No sugar.
5:27			*Injection begun into superficial branch of left femoral vein also.*		
5:30	45	8+		Alb.	No sugar.
5:45	70	25		Alb.	No sugar.
6:00	70	60		Alb.	No sugar.
6:05			Clear like H_2O,	Alb.	Trace sugar.
6:10			faintly acid.	Alb.	Sugar.†
6:15	65	72		Alb.	Sugar.†
6:30	55	35		Alb.	Sugar.†
6:45	65	55		Alb.	Much sugar.†
Total	505	280	The excretion of sugar continued for three hours when the animal died of air embolism. The air accidentally entered the injection tube while one of the cylinders was being refilled with salt solution.		
Total at end of exp...	900	605			

Experiment 3.

Injection fluid: $\frac{1}{6}$ molecular NaI.

Large male black and white rabbit. Fed on carrots for several days past in addition to barley and alfalfa hay. Urine obtained by catheterization.

Time.	Amount of salt solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.		
A.M.				
10:10	....	....	Tied to table.	
10:30	....	2	Yellow, turbid, faintly alk.	No sugar.
10:45	....	4	Yellow, turbid, faintly alk.	No sugar.
11:00	....	7	Pale yellow, slightly turbid, neutral, alb. *Injection of $\frac{1}{6}$ mol. NaI begun into superficial branch of right femoral vein.*	No sugar.
11:15	30	10	Pale yellow, slightly turbid, neutral.	No sugar.
11:30	25	10		No sugar.
11:45	50	10	Clear like H_2O, much alb. Further tests for alb. discontinued because of large amount of iodine set free on addition of acid to urine.	No sugar.
12:00	50	20		No sugar.
P.M.				
12:07				Trace sugar.
12:15	85	70	Clear like H_2O, faintly acid.	Sugar.†
12:22				Sugar.†
12:30	110	95		Sugar.†
12:45		70		Much sugar.†
1:00	50	65		Much sugar.
Total.....	400	350	The excretion of sugar continued two hours longer when the animal died. Death due to iodine poisoning (?).	
Total at end of exp.....	540	470		

Experiment 4.

Injection fluid: $\frac{1}{8}$ molecular $NaNO_3$.

Large female Belgian Hare. Fed carrots for several days past. Urine obtained by manual pressure over the bladder. Incontinence of the bladder developed and some urine was lost in this way. Whenever this occurred it is indicated by (+) after the amount of urine excreted.

Time.	Amount of salt solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.		
A.M.				
9:13	...	....	Tied down.	
9:15	....	1	Turbid, yellow, alk.	No sugar.
9:45	....	1	Turbid, yellow, alk. *Injection of $\frac{1}{8}$ mol. $NaNO_3$ begun into both ears.*	No sugar.
10:00	80	1	Clear, light yellow, faintly acid.	No sugar.
10:15	....	....		
10:22			One needle, which the animal has torn out of its ear by moving its head is transferred to superficial branch of right femoral vein.	
10:30	75	10	Clear, light yellow, faintly acid.	No sugar.
10:45	85	14+	Clear, light yellow, faintly acid. No alb.	No sugar.
11:00	90	18+	Clear, light yellow, faintly acid. Trace alb.	No sugar.
11:07				No sugar.
11:15	100	38+		Trace sugar.
11:22				Trace sugar.
11:30	90	58		Trace sugar.
11:37				Trace sugar.
11:45	150	54+	Clear like H_2O, faintly acid.	Sugar†, great increase
11:52				Sugar.†
12:00	95	110+		Much sugar.†
P.M.				
12:07			Trace alb.	Much sugar.
12:15	70	55+	Trace alb.	Much sugar.
Total	835	358+	The further course of this experiment need not be given here.	
Total at end of exp.	1470	1320+		

I tried next to see whether sodium citrate is able to bring about glycosuria more rapidly than sodium chloride. Two experiments sufficed to show that pure solutions of sodium citrate of a concentration approaching the osmotic pressure of the blood are rapidly fatal when only a few cubic centimeters are injected intravenously. I could not, therefore, compare directly the effects of a pure sodium citrate solution with those of a pure sodium chloride solution. That, however, sodium citrate acts more powerfully than sodium chloride in bringing about glycosuria is shown by the following facts.

In a number of animals I was unable to bring about glycosuria by the injection of a definite amount of a $\frac{1}{6}$ molecular NaCl solution during each fifteen minutes. In these animals glycosuria was produced, however, when a small amount of sodium citrate (about 2 c.c. $\frac{1}{8}$ molecular solution during each five minutes for half an hour) was injected by means of a hypodermic syringe into the rubber tubes *o* or *o,* (see diagram) carrying the sodium chloride solution into the animal. Again, when sodium citrate was given to animals which were already excreting small quantities of sugar under the influence of a sodium chloride solution the excretion of sugar was greatly increased.

V.—The Power of Calcium Chloride to Suppress the Glycosuria Produced by Sodium Salts.

The investigations of Loeb[7] have shown that the muscular twitchings produced by sodium salts can be inhibited by soluble calcium salts. My experiments on glycosuria show that *the excretion of sugar which is brought about by a pure sodium chloride solution can be stopped by the addition of calcium chloride to the sodium chloride solution.* If about 75–100 c.c. of a $\frac{1}{6}$ molecular NaCl solution are injected during each fifteen minutes into the circulation of a rabbit, *glycosuria develops* in the course of about two hours. *This glycosuria can be stopped* even when the same amount of fluid is injected into the animal during each fifteen minutes *by the injection of the following mixture: 25 c.c. $\frac{3}{8}$ molecular $CaCl_2$ + 975 c.c. $\frac{1}{6}$ molecular NaCl. After the excretion of sugar has been stopped by this means, it can be started again* by

[7] Loc. cit.

substituting for this mixture a pure $\frac{1}{6}$ molecular NaCl solution. In large, strong rabbits this can be done several times in succession.

A considerable latent period exists before the specific effects of the various salts show themselves. When the excretion of sugar has been started by a pure NaCl solution, for example, it does not cease for one or two hours after the injection of 975 c.c. $\frac{1}{6}$ molecular NaCl+25 c.c. $\frac{3}{8}$ molecular $CaCl_2$ is begun. After the excretion of sugar has once been stopped by this means it cannot be started again for an hour or two after the return to the pure NaCl solution.

What has been said in the preceding paragraphs is apparently true also of the glycosuria produced by pure solutions of NaBr, NaI, and $NaNO_3$. I have succeeded in stopping the glycosuria produced by pure $\frac{1}{6}$ molecular solutions of these salts by substituting for the pure solutions a mixture of 25 c.c. $\frac{3}{8}$ molecular $CaCl_2$ with 975 c.c. of the sodium salt solutions long before the amount of sugar excreted in the urine could possibly have equaled the total amount of sugar contained in the body. In all the experiments made thus far, however, the specific poisonous effects of the anions of these sodium salts have always killed the animal before I could obtain sugar in the urine a second time.

The following two experiments will serve to illustrate how glycosuria can be produced in rabbits by a pure sodium chloride solution, and how this glycosuria can be suppressed by substituting for the pure sodium chloride solution a mixture of sodium chloride with calcium chloride.

Experiment 5.

Injection fluid: $\frac{1}{6}$ molecular NaCl alternating with 975 c.c. $\frac{1}{6}$ molecular NaCl+ 25 c.c. $\frac{3}{8}$ molecular $CaCl_2$.

Large black and white female rabbit. Fed on carrots for several days past. Urine obtained by catheterization.

Time.	Amount of salt solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.		
P.M.				
3:45	....	....	Animal tied to table.	
4:00	....	....	*Injection of $\frac{1}{6}$ mol. NaCl begun into left ear.*	
4:15	100	....		
4:30	95	8	Slightly turbid, yellow, alk.	No sugar.
4:45	100	20	Clear, pale yellow, neutral. No alb.	No sugar.
5:00	100	15	Clear, pale yellow, neutral. No alb	No sugar.
5:15	105	23	*Injection begun into superficial branch of right femoral vein also.*	No sugar.
5:30	115	15+	No alb.	No sugar.
5:45	200?	50+	No alb.	No sugar.
6:00	85	80		No sugar.
6:15	100	63	Clear like H_2O, No alb.	No sugar.
6:30	95	42	neutral.	Trace sugar.
6:37				Trace sugar.
6:45	115	58		Sugar.†
6:52	35			Sugar, great increase†
			Injection fluid changed!! Injection of 975 c.c. $\frac{1}{6}$ mol. NaCl + 25 c.c. $\frac{3}{8}$ mol. $CaCl_2$ begun into superficial branch of right femoral vein. Injection into ear stopped.	
7:00	75	84	No alb.	Much sugar.†
7:07			No alb.	Much sugar.
7:15	50	54	Clear like H_2O,	Sugar.↓
7:22			faintly acid.	Sugar.↓
7:30	85	45	No alb.	Sugar, marked fall.↓
7:37				Trace sugar.↓
7:45	50	55		Trace sugar.
			Injection fluid changed!! Injection with $\frac{1}{6}$ mol. NaCl recommenced.	

7:52			No alb.	Trace sugar.
8:00	100	58		Trace sugar.
8:07				Trace sugar.
8:15	90	44		Trace sugar.
8:22				Trace sugar.
8:30	75	55		Trace sugar.
8:37				Trace sugar.
8:45	100	54		Trace sugar.
8:52				Trace sugar.
9:00	85	60		Trace sugar.
9:07			Clear like H_2O,	Trace sugar.
9:15	85	65	faintly acid.	Trace sugar.
9:22				Trace sugar.
9:30	80	50		Trace sugar.
9:37				Trace sugar.
9:45	90	57		Trace sugar.
9:52				No sugar.
10:00	100	58		No sugar.
10:07				No sugar.
10:15	90	68		No sugar.
10:22				No sugar.
10:30	60	56	Injected 1 c.c. $\frac{1}{8}$ mol. Sodium Citrate into tube o (see diagram). No alb.	No sugar.
10:37				No sugar,
10:45	60	60		No sugar.
10:52			Injected 1 c.c. $\frac{1}{8}$ mol. Sodium	Trace sugar.
11:00	65	55+	Citrate into tube every $7\frac{1}{2}$	Trace sugar.
11:07			minutes.	
11:15	75	5		Trace sugar.
11:25	?		*Injection fluid changed!! Injection with 975 c.c. $\frac{1}{6}$ mol. NaCl + 25 c.c. $\frac{3}{8}$ molecular $CaCl_2$ begun a second time.*	
11:30	15	5		Trace sugar.
11:37				Sugar, great increase†
11:45	80	5		Sugar† over 0.1%.
11:52			No alb.	Sugar over 0.1%.
12:00 A.M.	70	11		Sugar over 0.1%.
12:07			Clear like H_2O,	Sugar over 0.1%.
12:15	80	24	faintly acid. No alb.	Sugar over 0.1%.
12:22				Sugar over 0.1%.
12:30	40	39+		Sugar 0.1%.
12:37				Sugar 0.1%.
12:45	50	55		Sugar 0.1%.
12:52				Sugar; marked fall.‡
1:00	40	60		Sugar; marked fall.‡
1:07			Hypodermic inj. of 2 c.c. 1% morphine sulphate.	Sugar.
1:15	40	50		Trace sugar.
1:22				No sugar.
1:30	75	47	Hypodermic inj. of 2 c.c. 1% morphine sulphate.	No sugar.
1:37			Clear like H_2O, faintly acid.	No sugar.
1:45	?	50+	*Injection fluid changed!! Injection with $\frac{1}{3}$ mol. NaCl begun for third time.*	No sugar.
2:00		47	Animal is in good condition. Left for over night.	No sugar.

Experiment 5.—(Continued.)

Next day P.M.				
1:00	....	....	190 c.c. slightly bloody urine collected from dish provided to receive it during the night.	Sugar over 0.15%.
			60 c.c. slightly bloody urine removed from bladder.	Sugar over 0.15%.
			Animal, still in fair condition, removed from table and killed. The injection apparatus blew up through the night, and water flowed into the graduated cylinders. The amount of $\frac{1}{6}$ mol. NaCl solution which passed into the animal could therefore not be determined.	

Experiment 6.

Injection fluid: $\frac{1}{6}$ molecular NaCl alternating with 975 c.c. $\frac{1}{6}$ molecular NaCl + 25 c.c. $\frac{3}{8}$ molecular $CaCl_2$.

Large male Belgian Hare. Fed on carrots over night. Urine obtained by catheterization.

Time	Amount of salt solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.		
A.M.				
11:43	....	....	Animal tied to table.	
11:45	....	5	Turbid, yellow, alkaline. *Injection of $\frac{1}{6}$ mol. NaCl begun into both ears.*	No sugar.
12:00	100	6	Urine becoming clearer, faintly acid.	No sugar.
P.M.				
12:15	100	14		No sugar.
12:30	90	20		No sugar.
12:45	55	25		No sugar.
1:00	65	44	Clear like H_2O, faintly acid.	No sugar.
1:15	60	40		No sugar.
1:30	120	37		No sugar.
1:45	130	55		Trace sugar.
1:52	20		*Injection fluid changed!! Injection begun with 975 c.c. $\frac{1}{6}$ mol. NaCl + 25 c.c. $\frac{3}{8}$ mol. $CaCl_2$.*	Sugar over 0.025%.†
2:00	80	100		Sugar over 0.035%.†
2:07				Sugar over 0.05%.†
2:15	110	128		Sugar over 0.05%.
2:22				Sugar over 0.05%.
2:30	110	97		Sugar 0.05%.
2:37			Clear like H_2O, faintly acid.	Sugar; marked fall; 0.03%.‡
2:45	80	95		Sugar; marked fall; less than 0.25%.‡
2:52				Trace sugar.
3:00	90	95		Trace sugar.
3:04	?		*Injection fluid changed!! Injection with $\frac{1}{6}$ mol. NaCl recommenced.*	
3:07				Trace sugar.
3:15	80	105		Trace sugar.
3:22				Trace sugar.
3:30	80	87	Clear like H_2O, faintly acid.	Trace sugar.
3:37				No sugar.
3:45	80	63		No sugar.
3:52				No sugar.
4:00	40	50+	Slightly bloody, acid.	No sugar.
4:07				No sugar.
4:15	85	65		Trace sugar.

Experiment 6.—(Continued.)

Time.	Amount of salt solution in c.c. injected during last interval of time.	Amount of urine in c.c. excreted during last interval of time.		
P.M.				
4:22				Trace sugar.
4:30	85	55		Trace sugar.
4:37				Sugar.†
4:45	100	60	Clear like H_2O, faintly acid.	Sugar; great increase; 0.025%.†
4:52				Sugar 0.025%.
4:55	50?	....	*Injection fluid changed!! Injection with 975 c.c. $\frac{1}{8}$ mol. NaCl + 25 c.c. $\frac{3}{8}$ mol. $CaCl_2$ begun a second time.*	
5:00	20	60		Sugar.
5:07				Sugar.
5:15	100	45		
5:30	125	50		Sugar.
5:37				Sugar.
5:45	120	72		Sugar; 0.025%.
5:52			Slightly bloody, acid.	Sugar over 0.025%.†
6:00	75	52		Sugar 0.05%.†
6:07				Trace sugar; great fall.↓
6:15	65	32		No sugar.
6:22				No sugar.
6:30	90	14		No sugar.
6:32			*Injection fluid changed!! Injection with $\frac{1}{8}$ mol. NaCl begun a third time.*	
6:37				No sugar.
6:45	85	8	Slightly bloody, acid.	No sugar.
7:00	110	6		No sugar.
7:15	100	4		No sugar.
7:30	60	3		No sugar.
7:45	70	3	Very bloody, acid.	No sugar.
8:00	100	5		No sugar.
8:05				
8:15	60	15	Injected 1 c.c. $\frac{1}{8}$ mol. Na Citrate every 5 minutes into tube o (see diagram).	Trace sugar.
8:30	70	20		Trace sugar.
8:45	100	20	Urine is turbid, bloody, acid.	Sugar.†
9:00	100	15		Sugar.
9:15	65	4		Sugar.
			Animal dies. The peritoneal cavity and the intestines are full of liquid; there is great oedema of the intestinal walls and of the body generally.	

Not in all cases was I able to stop the glycosuria produced by the infusion of pure solutions of sodium salts by substituting for these a mixture of sodium salt with calcium chloride. In three experiments which I performed I was unable to stop, by the addition of calcium chloride, the excretion of sugar which had been started by the infusion of a $\frac{1}{6}$ molecular NaCl solution. It is possible that the glycosuria in these animals was due to some other circumstance besides the infusion of the sodium chloride solution. One of the animals had received morphine, which is itself able to produce glycosuria according to Külz. In the second case I had succeeded in producing a heavy glycosuria (over 0.1%) by the injection of $\frac{1}{6}$ molecular NaCl. The amount of sugar was reduced to a mere trace by substituting for the pure NaCl solution a mixture of NaCl with $CaCl_2$. I obtained sugar above 0.1% a second time by returning to the pure NaCl solution. This excretion of sugar was again reduced to less than 0.025% by injecting a mixture of NaCl and $CaCl_2$. The sugar then rose a third time in spite of the continued injection of the mixture of NaCl and $CaCl_2$. This uncontrolable excretion of sugar started shortly after the animal dislocated its right shoulder. For the third I can find no explanation at all. I thought it possible that the simple process of tying the animal fast to the table might bring about glycosuria in certain of the animals. Böhm and Hoffmann[8] state that sugar always appeared in the urine of cats when these animals were fastened to the animal board. This does not seem to be true of rabbits, however, for I tied six of these animals to the table and left them in this position for 24–36 hours. In none of them did sugar appear in the urine.

VI.—On the Relation Between the Amount of Salt Solution Injected and the Amount of Urine Excreted.

When $\frac{1}{6}$ molecular sodium chloride is injected intravenously into rabbits the quantity of urine excreted is, for longer periods of time, about equal to the amount of salt solution injected. During the first hour or two after the beginning of the injection the quantity of urine excreted lies below the amount of salt solu-

[8] Böhm and Hoffmann. Cited from Naunyn, B.: Der Diabetes Melitus, Wien, 1898.

tion injected. After this, however, the amount of urine excreted parallels roughly the amount of salt solution injected. Often after an experiment has lasted a number of hours the total amount of urine excreted is just equal to the total amount of salt solution which has been injected. More frequently the difference which exists between the amount of urine excreted during the first hour or two after the beginning of the injection and the amount of salt solution injected during the same time, is maintained throughout the experiment.

The following table gives in parallel columns the amount of $\frac{1}{6}$ molecular NaCl solution injected and the amount of urine excreted during the same time in seven experiments.

	Total amount of salt solution injected.	Total amount of urine excreted.
(Exp. 1)	1185	1225
	1125	1016
	1005	910
	1235	1198
	835	799
	300	240
	2415	1988

When more concentrated solutions of sodium chloride than $\frac{1}{6}$ molecular are injected into rabbits more urine is excreted than salt solution is injected. When less concentrated solutions of sodium chloride than $\frac{1}{6}$ molecular are injected into rabbits less urine is usually excreted than salt solution is injected. Animals in which this happens develop ascites, the intestines become filled with water and a general oedema supervenes. These symptoms were caused to disappear in two experiments which I performed by substituting for the weak sodium chloride solution a more concentrated one. These facts are indicated in the following table:

	Total amount of salt solution injected.	Total amount of urine excreted.
Gram m NaCl	235	317
$\frac{1}{2}$ m "	130	160
$\frac{1}{4}$ m NaCl	1130	594
" "	650	427

$\frac{1}{6}$ molecular sodium bromide, sodium iodide, and sodium nitrate solutions all have specific poisonous effects, in consequence of which less urine is excreted than salt solution is

injected. Sodium nitrate seems of all these solutions to be the most harmless in this respect. The following table will serve to illustrate these facts.

		Total amount of salt solution injected.	Total amount of urine excreted.
(Exp. 2)	$\frac{1}{6}$ m NaBr	900	605
	" "	1025	797
(Exp. 3)	$\frac{1}{6}$ m NaI	540	470
(Exp. 4)	$\frac{1}{6}$ m $NaNO_3$	1470	1320

In long continued experiments, no matter what the nature of the solution which is injected, the functions of the kidney apparently become impaired and much less urine is excreted than salt solution is injected. Animals in which this happens develop ascites; the walls of the intestine become greatly oedematous and the lumina of the intestines become filled with water. If the animal does not die at this stage, which is usually the case, the tissues of the body as a whole also become somewhat oedematous.

During the course of an experiment a number of circumstances may alter *temporarily* the amount of urine excreted in the unit of time, even though the amount of salt solution passing into the animal is kept constant. Whenever a mixture of $\frac{1}{6}$ molecular NaCl, NaBr, NaI, or $NaNO_3$ with $CaCl_2$ is suddenly substituted for a pure $\frac{1}{6}$ molecular solution of these sodium salts, a *temporary fall occurs in the amount of urine excreted*, even though the amount of liquid passing into the body in the unit of time is kept the same. This decrease in the amount of urine lasts usually about an hour, when the quantity excreted mounts to its old level in spite of the continued infusion of the mixture of the sodium salt with calcium chloride. This is analogous to the observation of MacCallum,[9] who found that the intravenous injection of calcium chloride decreases the rapidity of the normal secretion of urine.

The addition of sodium citrate to a pure solution of NaCl, NaBr, NaI, or $NaNO_3$ usually *causes a temporary rise in the amount of urine excreted*. This effect, too, passes off within an hour and the amount of urine then drops down to its old level, even though the injection of sodium citrate is continued. Three

[9] MacCallum, J. B.: University of California Publications. Physiology, 1904. I, p. 81.

times I noticed *a marked decrease in the amount of urine excreted when sodium citrate was injected* into a rabbit in addition to the pure sodium chloride solution. The explanation of this action of sodium citrate is probably analogous to that of barium chloride. MacCallum[10] found that while small doses of barium chloride increase the secretion of urine, larger doses cause anuria, apparently by bringing about a contraction of the musculature of the ureters.

Finally, it must be added that *the amount of urine and the amount of sugar excreted bear no relation to each other*, and vary independently of each other. An increase in the amount of urine excreted may be accompanied by either an increase or a decrease in the percentage of sugar contained in the urine. Similarly a fall in the excretion of urine may be accompanied by either an increase or a decrease in the concentration of the sugar in the urine.

Albuminuria is a frequent accompaniment of the glycosuria. I thought at first that the two always went together, but I now have a number of records of experiments in which glycosuria developed, but albumin never once appeared in the urine. A number of times the normal urine obtained from the bladders of animals freshly brought into the laboratory contained albumin. More frequently albuminuria did not evidence itself until the injection of salt solution had been continued for several hours. Sometimes the albuminuria ceased after having been present for a number of hours. I used the nitric acid test to detect the presence of albumin in the urine.

VII. Conclusions.

1. The injection of 75–100 c.c. of a $\frac{1}{6}$ molecular NaCl, NaBr, NaI, or $NaNO_3$ solution during each fifteen minutes into the circulation of rabbits brings about a polyuria and a glycosuria. The polyuria evidences itself in ten to fifteen minutes after the beginning of the injection, and lasts as long as the injection is continued (twenty-seven hours). The glycosuria does not evidence itself until about two hours after the beginning of the injection, and and lasts six to eight hours. The amount of

[10] MacCallum, J. B.: Loc. cit.

sugar excreted in the urine is at first small, rapidly increases to a maximum and then gradually falls to zero. The amount of sugar excreted when the excretion is at its height lies above 0.25%. When the excretion of sugar has fallen to zero, continued infusion of the sodium salts causes no more sugar to appear in the urine, even though the polyuria continues as before.

2. The fact that the amount of sugar excreted in the urine rarely exceeds 0.25% does not adequately express the severity of the diabetes. The fact must be considered that under the conditions of the experiment about twenty-five times as much urine is excreted in the unit of time as normally, through the infusion of large amounts of water into the circulation. Did this dilution of the urine not occur, the amount of sugar in the urine would at times exceed 6%.

3. If a less concentrated solution of NaCl than a $\frac{1}{6}$ molecular is injected intravenously, polyuria is still brought about, but glycosuria appears not at all or only much later than when the same quantity of a $\frac{1}{6}$ molecular NaCl solution is injected. If a more concentrated solution of NaCl is injected, glycosuria appears the more rapidly the greater the concentration of the NaCl solution.

4. If glycosuria is started in an animal by the intravenous injection of pure solutions of NaCl, NaBr, NaI, or $NaNO_3$ the excretion of sugar can be markedly lessened or suppressed entirely by substituting for the $\frac{1}{6}$ molecular NaCl, NaBr, NaI, or $NaNO_3$ solutions a mixture of one of these with $CaCl_2$ (for example, 975 c.c. $\frac{1}{6}$ molecular NaCl + 25 c.c $\frac{3}{8}$ molecular $CaCl_2$).

5. Animals which will excrete no sugar in their urine when a certain amount of $\frac{1}{6}$ molecular NaCl alone is injected can usually be caused to excrete sugar by substituting for the pure NaCl solution a mixture of $\frac{1}{6}$ molecular NaCl with sodium citrate. When sodium citrate is given to animals which are already excreting a small amount of sugar, the excretion of sugar is increased.

6. The animals seem to vary in regard to the ease with which glycosuria can be produced in them. Some rabbits excrete sugar in their urine sooner and after the injection of

smaller amounts of $\frac{1}{6}$ molecular NaCl, NaBr, NaI, or $NaNO_3$ solutions than do others. No rabbits have been found in which glycosuria could not be produced by $\frac{1}{6}$ molecular solutions of these sodium salts if injected in sufficient amounts. Some difference seems to exist also in regard to the ease with which the excretion of sugar can be suppressed in different animals. Some animals cease to excrete sugar sooner and after smaller doses of $CaCl_2$ than others.

7. A considerable latent period exists before the specific effects of the various salts show themselves. When the excretion of sugar has been started by a pure NaCl solution, for example, it does not cease for an hour or two after the injection of 975 c.c. $\frac{1}{6}$ molecular NaCl + 25 c.c. $\frac{3}{8}$ molecular $CaCl_2$ is begun. After the excretion of sugar has once been stopped by this means, it cannot be started again for an hour or two after the return to the pure NaCl solution.

8. For longer intervals of time the amount of urine excreted is about equal to the amount of $\frac{1}{6}$ molecular NaCl solution injected. Sodium chloride solutions of a higher concentration than this cause more urine to be excreted than salt solution is injected. When equimolecular NaBr, NaI or $NaNO_3$ solutions are injected less urine is usually excreted than salt solution is injected. This is true also of sodium chloride solutions of a lower concentration than $\frac{1}{6}$ molecular. In long continued experiments, no matter what the nature of the injection fluid, the kidneys apparently become impaired after a time, and less urine is excreted than salt solution is injected. Animals in which this happens develop ascites; the intestinal walls become oedematous and the intestines themselves become filled with liquid; later oedema of the body as a whole may develop.

9. The substitution of a mixture of NaCl, NaBr, NaI or $NaNO_3$ with $CaCl_2$ for a pure solution of one of these sodium salts is followed by a temporary fall in the amount of urine excreted in the unit of time.

10. The substitution of a mixture of NaCl, NaBr, NaI or $NaNO_3$ with sodium citrate for a pure solution of NaCl, NaBr, NaI or $NaNO_3$ is usually followed by a temporary rise in the amount of urine excreted in the unit of time. Sometimes sodium

citrate brings about a fall in the amount of urine excreted. The latter is probably due to a constriction of the ureters.

11. The amount of urine and the amount of sugar excreted bear no relation to each other and vary independently of each other.

12. Albuminuria is a frequent, though not constant, accompaniment of the glycosuria.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 13, pp. 115-123 March 25, 1904

THE INFLUENCE OF SALINE PURGATIVES ON LOOPS OF INTESTINE REMOVED FROM THE BODY.

BY

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It has been shown in previous papers[1] that the administration of solutions of certain salts including the so-called saline purgatives causes in rabbits and dogs not only increased peristaltic movements of the intestine, but also an increased secretion of fluid into the intestine. This was found to be true not only when the purgatives were administered subcutaneously or intravenously, but also when the saline solutions were applied locally to the peritoneal surfaces of the intestine. It was shown that the administration of solutions of calcium chloride by any of these methods counteracts the effect of the purgative salts; and it was further pointed out that these actions are analogous to the production and suppression of twitchings in voluntary muscle described by Loeb.[2]

The following is a description of a number of experiments made on loops of the intestine entirely removed from the body in order to determine the action of the saline solutions on the isolated intestine, more particularly with regard to the production of increased secretion of fluid into the intestine.

[1] MacCallum, J. B.; University of California Publications, Physiology, 1903, i, p. 5. American Journal of Physiology, 1903, x, p. 101, and 1904, x, p. 259.

[2] Loeb, J.; Festschrift für Fick 1899; Archiv für die gesammte Physiologie, 1902, xci, p. 248.

Methods.

In these experiments only rabbits were made use of. Morphine was employed as an anaesthetic, 4–5 c.c. 1% solution of the hydrochlorate being administered. The abdominal cavity was opened and the blood vessels supplying the loop selected were carefully tied off. Two pairs of ligatures were then placed around the intestine so that the loop was properly isolated. The intestine was then cut between each pair of ligatures and the mesentery divided between the blood vessel ligatures and the intestine. In this way the loop could be taken from the body without injuring it, and without causing the animal to lose more than a few drops of blood. The loop was then emptied by allowing the fluid to drain from one end while it was held up by the ligature of the other end. The open end was then religatured and the whole loop suspended in the solution to be tested. This was so carried out that both ends of the loop were above the surface of the solution. It was then quite impossible for any of the solution to enter the lumen through the ligatured ends. The solutions were kept in beakers in a water-bath at 39.5° C. The movements of the loops could be directly watched, and the amount of fluid secreted in a unit of time could be easily measured.

Experiments.

If a loop be removed from the intestine of a rabbit, and suspended in a solution of $\frac{m}{8}$ NaCl, active peristaltic movements will at once set in. These contractions are regular and resemble the peristalsis induced by the administration of a saline purgative to an animal with its intestine intact. If the loop be allowed to lie on the bottom of the vessel containing the solution it will writhe about in a peculiar worm like manner. These movements persist in varying intensity for a considerable time. No attempt was made to obtain a fluid in which the intestine could be kept for a long period of time. In pure $\frac{m}{8}$ NaCl solution however a loop frequently continues to move for 40 or 45 minutes. The movements become gradually more feeble and finally disappear. It is important of course that the solution should be kept at body temperature. That the loop however is not dead

when the movements cease is shown by the fact that it can be caused to exhibit active movements again by transferring it to a solution of $\frac{m}{8}$ NaCl containing a small quantity of $\frac{m}{8}$ $BaCl_2$. These movements last only a few minutes and the loop soon comes to rest again. If now it be transferred back to $\frac{m}{8}$ NaCl solution slight movements again make their appearance. I have made no attempt to work out the details of this phenomenon. It seems however to be analogous to the contact irritability which Loeb has described in the case of voluntary muscles.

When a loop is carefully emptied and suspended in a solution of $\frac{m}{8}$ NaCl so that the ligatured ends are above the level of the fluid, it is found that after a considerable time (20 to 40 minutes) it is still quite empty. If on the other hand a similar loop be suspended in $\frac{m}{2}$ NaCl solution it is found to contain after 15 to 20 minutes a small but distinct quantity of clear yellowish fluid resembling the normal intestinal juice. The quantity of the fluid varies with the length of the loop. A loop 27 cm. long suspended in $\frac{m}{2}$ NaCl for 20 minutes was found to contain 0.6 cc. fluid. A second loop 30 cm. long suspended in a similar solution for the same time contained 0.8 c.c. A control loop 30 cm. long suspended in $\frac{m}{8}$ NaCl was at the end of 20 minutes still quite empty.

In $\frac{m}{2}$ NaCl the muscular movements are somewhat different from those seen in loops placed in $\frac{m}{8}$ NaCl. In the stronger solution spasmodic contractions of considerable violence occur. Sometimes the loop contracts forcibly only once or twice and then remains quiet. The movements seldom continue in $\frac{m}{2}$ NaCl for longer than five minutes. The loops are not entirely killed by immersion for 20 minutes in $\frac{m}{2}$ NaCl for when they are transferred to $\frac{m}{8}$ NaCl solution containing $\frac{1}{25}$ of its volume of $\frac{m}{8}$ $BaCl_2$ active muscular movements immediately begin. Similar experiments were made with other salts. A loop 30 cm. long suspended in $\frac{m}{8}$ sodium citrate solution showed active peristaltic movements which continued for a considerable time, but not so long as those in the loops immersed in $\frac{m}{8}$ NaCl. After 20 minutes the loop was found to be still empty and no fluid appeared in it subsequently. A similar loop however 32 cm. long suspended in $\frac{m}{2}$ sodium citrate solution contained after 20 minutes 0.4 c.c. of

fluid. The muscular movements caused by this strong solution were similar to those produced by $\frac{m}{2}$ NaCl.

A loop suspended in $\frac{m}{8}$ Na_2SO_4 showed active peristaltic movements but after 20 minutes still contained no fluid. A similar loop suspended in $\frac{m}{2}$ Na_2SO_4 for 20 minutes was found to contain 1.5 cc. fluid at the end of that time though it was quite empty in the beginning.

A loop 28 cm. long suspended in $\frac{m}{8}$ NaF exhibited active peristaltic movements more convulsive in character than those produced by sodium chloride, citrate, or sulphate. These continued for less than 10 minutes. At the end of 20 minutes the loop was found to contain 0.8 cc. of a clear somewhat bloody fluid.

Loops suspended in $\frac{m}{8}$ NaCl containing $\frac{m}{8}$ $BaCl_2$ in the proportion of 50 to 1 or 70 to 1 showed very strong muscular contractions and well marked secretion of fluid. The muscular movements were always of the type which has already been described as characteristic of the action of barium. Violent local contractions and firm constrictions of the intestine together with strong peristaltic movements took place. The loops always contained a considerable amount of fluid after being suspended in this fluid. A loop 25 cm. long after 20 minutes contained 0.6 c.c.; a second loop 46 cm. long after the same length of time contained 0.8 c.c.; a third 60 cm. in length, in the same time secreted 1.2 c.c. fluid; a fourth 31 cm. long after 20 minutes contained 1.2 c.c.; a fifth 63 cm. long secreted in 20 minutes 1.6 c.c. fluid. A loop 32 cm. long suspended in pure $\frac{m}{8}$ $BaCl_2$ solution contained after 20 minutes 2.7 c.c. of bloody fluid. A number of other experiments with barium gave similar results. A distinct secretion of fluid was always brought about by suspending loops of the intestine in solutions containing barium chloride.

Loops placed in $\frac{m}{8}$ $CaCl_2$ showed no muscular movements whatever, nor did any fluid gather in the lumen. This was a marked contrast to the behavior of the loops in sodium citrate, chloride or sulphate, etc., or in barium chloride. Whereas the intestine was found to exhibit active peristaltic movements in $\frac{m}{8}$ NaCl or any of the purgative saline solutions described, it lay perfectly quiet in a solution of $\frac{m}{8}$ $CaCl_2$.

In a mixture of equal parts of $\frac{m}{8}$ NaCl and $\frac{m}{8}$ $CaCl_2$ solutions, loops of the intestine showed practically no peristaltic movements. Slight isolated twitchings of the muscle coats occasionally occurred, but the regular peristaltic movements characteristic of the action of the NaCl solution were quite counteracted by the presence of $CaCl_2$. No secretion of fluid took place into the intestine in this mixture. A loop suspended in a mixture of equal parts of $\frac{m}{2}$ NaCl and $\frac{m}{2}$ $CaCl_2$ showed slight muscular movements and no trace of fluid in the lumen after 20 minutes.

Loops suspended in mixtures of NaCl, $BaCl_2$ and $CaCl_2$ showed that the presence of calcium modified to a certain extent the action of the barium but did not entirely counteract its influence on either the muscular or the glandular tissue. A loop 25 cm. long placed in a mixture of 50 c.c. $\frac{m}{8}$ $CaCl_2$ and 1 c.c. $\frac{m}{8}$ $BaCl_2$ showed peculiar spasmodic contractions of the muscle quite different from the characteristic barium contractions. After 20 minutes a very small quantity of fluid, 0.2 c.c., was found in the loop. Other loops placed in the same mixture showed no secretion of fluid.

The results of a number of these experiments may be observed in the following table:

No.	Salt	Concentration of Solution	Time	Length of loop	Muscular movements and duration	Secretion of fluid
1	NaCl	$\frac{m}{8}$	40 min.	30 cm.	Active peristalsis 40 min.	0.0
2	"	"	20 "	23 "	Active peristalsis 40 min.	0.0
3	"	$\frac{m}{2}$	20 "	30 "	Strong contractions 5 min.	0.8 c.c. clear yellow fluid.
4	"	"	20 "	30 "	Strong contractions 5 min.	0.6 c.c. clear yellow fluid.
5	Sod. Citrate	$\frac{m}{8}$	20 "	20 "	Active peristalsis 20 min.	0.0
6	"	$\frac{m}{2}$	20 "	32 "	Violent contractions 2–3 m.	0.4 c.c. clear yellow fluid.
7	Na_2SO_4	$\frac{m}{8}$	20 "	33 "	Active peristalsis.	0.0
8	"	$\frac{m}{2}$	20 "	30 "	Strong contractions of short duration.	1.5 c.c. clear yellow fluid.
9	Na F	$\frac{m}{8}$	20 "	28 "	Strong contractions 10 min.	0.8 c.c. slightly bloody.

No.	Salt	Concentration of Solution	Time	Length of loop	Muscular movements and duration	Secretion of fluid
10	$NaCl$ $BaCl_2$	$\frac{m}{8}$–70 c.c. $\frac{m}{8}$– 1 c.c.	20 min.	25cm.	Active movements.	0.6 c.c. clear yellow fluid.
11	"	"	20 "	46 "	Active movements.	0.8 c.c.
12	"	"	20 "	60 "	Active movements.	1.2 c.c.
13	"	"	20 "	31 "	Active movements.	1.2 c.c.
14	$NaCl$ $BaCl_2$	$\frac{m}{8}$–50 c.c. $\frac{m}{8}$– 1 c.c.	1st 20 min. 2nd 20 "	63 "	Active movements.	1.6 c.c. 0.2 c.c.
15	$BaCl_2$	$\frac{m}{8}$–50 c.c.	1st 20 " 2nd 20 "	32 "	Violent contractions.	2.7 c.c. 0.2 c.c.
16	$NaCl$ $BaCl_2$	$\frac{m}{8}$–30 c.c. $\frac{m}{8}$–30 c.c.	20 min.	23 "	Violent contractions.	0.9 c.c.
17	$CaCl_2$	$\frac{m}{8}$	20 "	20 "	None.	None.
18	"	"	20 "	15 "	"	"
19	$CaCl_2$ $NaCl$	$\frac{m}{8}$–50 c.c. $\frac{m}{8}$–50 c.c.	20 "	10 "	"	"
20	$CaCl_2$ $NaCl$	$\frac{m}{2}$–50 c.c. $\frac{m}{2}$–50 c.c.	20 "	15 "	Slight movements not peristaltic in character.	"
21	$CaCl_2$ $NaCl$	$\frac{m}{1}$–50 c.c. $\frac{m}{1}$–50 c.c.	20 "	15 "	Slight movements not peristaltic in character.	Slight trace.
22	$NaCl$ $BaCl_2$ $CaCl_2$	$\frac{m}{8}$–30 c.c. $\frac{m}{8}$– 1 c.c. $\frac{m}{8}$–30 c.c.	20 "	35 "	Irregular contractions, not strong.	None.
23	$NaCl$ $BaCl_2$ $CaCl_2$	$\frac{m}{8}$–30 c.c. $\frac{m}{8}$– 1 c.c. $\frac{m}{8}$–30 c.c.	20 "	30 "	Irregular contractions, not strong.	0.2 c.c.
24	$BaCl_2$ $CaCl_2$	$\frac{m}{8}$– 1 c.c. $\frac{m}{8}$–50 c.c.	20 "	30 "	Irregular contractions.	0.2 c.c.

From these experiments it is obvious that the saline purgatives act on the intestine not only when it has its normal position and connections with the rest of the body, but also when it is entirely

isolated. The action of these salts on a loop of intestine removed from the body of a rabbit is entirely analogous to the influence which, as shown by Loeb, they exert on a gastrocnemius muscle removed from the body of a frog. The special anatomical relations however which exist between the glandular and muscular tissues in the intestine bring up several points of interest.

As I have already shown the action of a saline purgative consists of two parts, the increase in peristaltic movement, and the increase in the secretion of fluid into the intestine; or in other words the action on the muscle and that on the glandular tissue. In the experiments which have just been described the fact that these two separate actions exist side by side is made still more clear. For example, $\frac{m}{8}$ solutions of sodium chloride, sulphate, or citrate, cause well marked peristaltic movements, but give rise to no secretion of fluid. Stronger solutions on the other hand such as $\frac{m}{2}$ produce a distinct secretory activity. Thus a concentration of the salt which is sufficient to produce muscular activity is usually not sufficient to cause the glandular tissue to become active. One is tempted to conclude therefore that in the intestine it requires a stronger stimulus to produce secretory activity than it does to cause muscular movements. It is possible that this is true, but the anatomical relations also must be taken into consideration. The muscle coats lie immediately under the thin peritoneal layer through which the salts are absorbed; and it seems probable that in the experiments described the solutions reach the muscle much more easily and rapidly than they do the glandular tissue which is situated on the other side of the muscle and submucous layers. It might be quite possible by injecting a series of solutions into the blood vessels to determine a concentration at which increased muscular movements are brought about without increased secretory activity.

A certain amount of interest attaches also to the fact that the saline purgatives have the same action on a loop of the intestine which is entirely separated from its bloodvascular, and central nervous connections as they exert on the intestine of an intact animal. The possibility in the first place of the solutions acting entirely through the central nervous system is eliminated. It is possible that they have some influence on the central nervous

system, but from these experiments it seems probable that their main action is either on the glandular and muscular tissues themselves, or on the plexuses of Auerbach and Meissner in the intestinal walls.

The amount of fluid which may be secreted by a loop of intestine isolated from the body is limited by the absence of the blood supply. The loop as shown above secretes a certain amount of fluid in the first 10 to 20 minutes. If it is then emptied usually no more fluid appears. In some cases a very minute quantity is secreted after the first amount is removed. The secretion thus depends on the quantity of fluid which is contained in the intestinal walls at the time of its removal from the body. No fluid passes from the solution in which the loop is suspended into the lumen of the loop. No current is established through the walls from the outside inwards. It seems possible to supply the stimulus for secretion in the solution in which the loop is suspended; but it is not possible in this way to renew the fluid which the glandular tissues have secreted into the lumen. This can apparently be done only through the blood vessels.

The antagonism which is seen to exist in these experiments between the purgative salts and calcium is the same as that already described by Loeb in their action on the voluntary muscle of the frog, and confirmed by myself in experiments on the intestine and kidney. The fact that a loop of the intestine which shows active peristaltic movements in $\frac{m}{8}$ NaCl solution lies perfectly quiet in a mixture of equal parts of $\frac{m}{8}$ NaCl and $\frac{m}{8}$ $CaCl_2$ suggests a practical application of this relation. It is often found that in the administration of rectal infusions of NaCl solution peristaltic movements are often produced which make it difficult to maintain the solution in the rectum until it is absorbed. It seems probable from these experiments that this difficulty might be obviated by the addition of small quantities of $CaCl_2$ to the NaCl solution. The only thing to be watched in the administration of calcium is the possible suppression of urine. As shown in a recent paper[1] calcium acts as a powerful antidiuretic, decreasing markedly the activity of the kidneys.

[1] MacCallum, J. B.; University or California Publications, Physiology, vol. i, No. 10, p. 81, 1904.

Conclusions.

1. When a loop of the small intestine of a rabbit is removed from the body, emptied, and suspended in $\frac{m}{8}$ NaCl solution containing $\frac{1}{70}$—$\frac{1}{50}$ of its volume of $\frac{m}{8}$ $BaCl_2$ in such a way that the ligatured ends are above the surface of the fluid, it is found to contain after 15 to 20 minutes a measurable quantity of fluid resembling the normal intestinal juice. Peristaltic movements characteristic of the action of barium also appear in the loop.

2. A loop similarly immersed in $\frac{m}{8}$ sodium chloride, sulphate or citrate shows active peristaltic movements, but contains no fluid after 15 to 20 minutes. If it is suspended however in a $\frac{m}{2}$ solution of one of these salts a measurable quantity of fluid collects in the loop. A loop emptied and suspended in $\frac{m}{8}$ Na F is found to contain a measurable quantity of fluid after 15 to 20 minutes.

3. It is possible in this way to produce peristaltic movements of the intestine by solutions whose concentration is not great enough to cause increased secretory activity. It is further seen that both peristaltic movements and the secretion of fluid into the intestine may be brought about in a loop which is entirely separated from the central nervous system, and is not supplied with blood.

4. The secretion of fluid takes place only in the first 15 to 20 minutes after the immersion of the loop in the saline solution, and cannot be made to continue after this.

5. Loops placed in $\frac{m}{8}$ $CaCl_2$ show no peristaltic movements and no secretory activity. Calcium chloride added to any of the solutions described above counteracts to a certain extent their action not only on the muscle, but also in the glandular tissue.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 14, pp. 125-137 March 30, 1904

THE SECRETION OF SUGAR INTO THE INTESTINE CAUSED BY INTRAVENOUS SALINE INFUSIONS.

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The experiments described below may be considered in the following parts:

1. The production of a largely increased secretion of fluid into the intestine by the intravenous injection of considerable quantities of $\frac{m}{8}$—$\frac{m}{6}$ NaCl solution, a condition analogous to the polyuria similarly produced.

2. The secretion of considerable quantities of sugar into the intestine produced by the injection of $\frac{m}{6}$ NaCl solution into the blood, analogous to the production of glycosuria by the same means.

3. The secretion of sugar into the stomach under similar circumstances.

4. The secretion of urea into the alimentary canal.

In connection with these problems also the question arises as to the extent to which the intestine acts as a supplementary excretory organ, and how far it can be made to excrete substances which are, under the same circumstances, constituents of the urine. It is interesting to enquire also how great the similarity is between the nature of the secretory activity in the intestine and that in the kidney

Methods.

In all these experiments rabbits were used which weighed between 1500 and 2000 grams. Morphine was used as an anaesthetic, 5 c.c. 1% solution being given subcutaneously. The operations were done rapidly and the animals protected as much as possible from loss of heat. Grammolecular solutions of the salts were prepared and the proper dilutions made from these. The infusions were made by means of an injection apparatus, such as is ordinarily used for this purpose, consisting of a pressure bottle connected with the vessel containing the salt solution, which in turn was connected with a long rubber tube coiled about in a vessel of water kept constantly several degrees above the body temperature. The fluid was introduced into the marginal vein of the ear through a hypodermic needle and reached the blood at approximately body temperature.

The left kidney was removed from the circulation by merely passing an aneurism needle under the vessels and ligating them with a thread. The ligation of the right renal vessels, however, is more difficult, and it was found that the best procedure consisted in freeing the kidney from the peritoneum and passing a loop of thread over it. This thread could then be securely tied around the vessels. The placing of cannulae in the intestinal loops requires no special description. It is important, however, to touch the loops as little as possible and to take all possible precautions against their becoming cold. The intestinal cannulae should be large with wide open mouths. By placing such a cannula in the pyloric opening the gastric juice may be collected without difficulty.

The ordinary methods of analysis were employed in examining the fluids secreted by the stomach, intestine, and kidneys. Qualitative tests for sugar were made by either Trommer's or Fehling's method. For quantitative estimations the ordinary method of titration using measured quantities of Fehling's copper sulphate solution was made use of. Urea was recognized by all the well known tests. It is important to make the sugar, and also the urea, determinations immediately after the fluid is obtained from the intestine since the sugar becomes rapidly fer-

mented, and the urea quickly breaks up into other compounds. It is important also to use well fed animals for such experiments since the sugar of the body diminishes when the rabbit is starved.

I.—The Increased Secretion of Fluid Into the Intestine Caused by Intravenous Injections of NaCl Solution.

It has been observed by several investigators that a quantity of fluid enters the intestine during the intravenous injection of normal salt solution. According to Dastre and Loye[1] the fluid injected into the veins is largely eliminated by the kidneys, although these organs may be assisted in this function by the salivary glands and the intestine. These authors have observed that in rabbits a certain amount of yellowish fluid is often present in the intestine as well as in the pleural and peritoneal cavities after the injection of large quantities of salt solution. They state that when the fluid is eliminated by the intestine a diarrhoea results which may be so pronounced that a clear fluid emerges from the rectum. Knoll[2] also mentions the production of diarrhoea by the infusion of large quantities of normal NaCl solution.

Magnus[3] in his work on the production of oedema of the skin by intravenous infusions of salt solutions, shows in his tables that a certain amount of fluid is eliminated by the intestine after the kidneys have been removed. A rabbit after double nephrectomy was infused with 1500 c.c. normal salt solution; 200 g. were eliminated by the intestine. In a second rabbit 1010 c.c. of fluid were injected into the veins and 60 g. were eliminated by the intestine. In a dog 1760 c.c. were injected and 160 g. eliminated by the intestine.

I have made a number of experiments in which the amount of fluid secreted by the intestine during the intravenous injection of large quantities of NaCl solution was determined not only when the kidneys had been removed from the circulation, but also when they were active. As shown by the following experiment, the fluid secreted by the intestine in the absence of the kidneys increases rapidly when $\frac{m}{8}$ NaCl solution is injected into the blood.

[1] Arch. de physiol. norm. et path. 4 e série, 2, 1888, p. 93; 5 e série, 1, 1889, p. 253.
[2] Arch. für exp. Path. und Pharmakol. Bd. xxxvi, S, 293. 1895.
[3] Arch. für exp. Path. und Pharmakol. Bd. xlii, S. 250, 1889.

Without such an injection or other stimulus very little intestinal juice can be gathered in a time as short as that occupied by the experiments. In a rabbit's small intestine there is usually found between 5 and 10 c.c. fluid—often much less.

Exp. 1. Rabbit. The blood vessels to the kidneys were tied off and two cannulæ were put into the intestine, one 35 cm. from the pylorus and the other in the lower part of the ileum. Each loop was isolated by ligatures. The upper one was about 30 cm. long and the lower one 42 cm. The upper loop in the beginning contained 5 c.c. fluid, which were removed. The lower loop was empty. During the first hour 100 c.c. $\frac{m}{8}$ NaCl were forced into the blood and 5.2 c.c. fluid appeared in the upper loop and nothing in the lower loop. During the second hour 240 c.c. NaCl solution were injected and 5.4 c.c. fluid appeared in the upper loop and 13.6 c.c. in the lower loop. During the third hour 160 c.c. NaCl solution were introduced; 6.6 c.c. fluid appeared in the upper loop and 15.5 c.c. in the lower. The infusion was then stopped and 26 c.c. were found in the part of the small intestine not included in the loops. The total amount thus secreted by the intestine in three hours was 72.3 c.c. This is 14.46% of the quantity injected.

Exp. 2. In another rabbit with kidneys removed 470 c.c. ^{m} NaCl solution were injected during three hours, and 78 c.c. fluid obtained from the intestine, which is about 16.6% of the quantity injected.

Exp. 3. In a rabbit in which the kidneys were left intact 547 c.c. $\frac{m}{6}$ NaCl solution were injected into the blood. 49.5 c.c. were secreted in 3 hours and 30 minutes by the intestine. This is about 9% of the total quantity of fluid. The quantity of fluid secreted into the loop in the first half hour was 1.8 c.c.; in the third half hour, 2.9 c.c.; in the fifth half hour, 6.6 c.c.

Exp. 4. In a second rabbit in which the kidneys were untouched, the quantity of $\frac{m}{8}$ NaCl solution introduced was 390 c.c; about 40 c.c. of fluid were obtained during three hours from the intestine. This is 10.25% of the amount injected. In the first hour 5 c.c. were secreted into the loop; in the second hour 7 c.c., and in the third hour 11.1 c.c. In the rest of the small intestine 17 c.c. were found.

It is clear from these experiments that *the intestinal secretion is remarkably increased by the intravenous injection of* $\frac{m}{8} - \frac{m}{6}$ *NaCl solutions*. The quantity is somewhat greater when the kidneys are removed. *The action of such infusions on the intestines is, however, quite similar to their influence on the kidneys.* In the kidneys polyuria is produced, and in the intestine an increase in secretory activity which is entirely similar to this phenomenon. The action of NaCl injections is the same as that exerted on the intestine by the saline purgatives,[1] among which NaCl must be classed. This condition of increased secretory activity in the intestines produced by infusions of NaCl and by saline purgatives is so similar to the production of polyuria by the same procedure that one is tempted to emphasize the analogy by terming it intestinal polyuria. The diuretic action of many other salts, such as Na_2SO_4, $BaCl_2$, etc., on the kidney is so similar to the action of the same salts in producing increased secretion of fluid into the intestines that one is also tempted to speak of intestinal diuresis. These terms, although they are introduced here only to show the analogy which exists between the activities of the kidney and the intestine, have a greater significance when we know, as will be shown below, that some of the substances secreted by the two organs are identical; and that the urine and the intestinal juice are in many ways similar. With the exception of the production of increased peristalsis in the intestine, there seems to be little difference between the action of a saline purgative on the intestine and that of a saline diuretic on the kidney. In most cases the salts are identical; all the saline purgatives act also as diuretics; one of the most powerful purgatives, $BaCl_2$, is also one of the most powerful diuretics. The production of increased activity in glandular tissue is a part both of diuresis and of purgation; and, as I have shown in previous publications, calcium inhibits the secretion of fluid into the intestine as well as the secretion of urine.

In the injection of considerable quantities of $\frac{m}{8} - \frac{m}{6}$ NaCl solution more fluid in general is excreted into the intestine when

[1] J. B. MacCallum. American Journal of Physiology, 1903, X, p. 101: 1904, X, p. 259. University of California Publications, Physiology, Vol. I, p. 81, 1904; Vol. I, p. 115, 1904.

the kidneys have been removed than when they are present. This is relatively unimportant, however, since the same conditions can be brought about otherwise, namely, by increasing the rapidity of the injection when the kidneys are intact. Just as is the case with the secretion of urine, the fluid secreted by the intestines varies roughly with the rapidity of the injection. The removal of the kidneys is in a sense equivalent to increasing this rapidity.

II.—The Secretion of Sugar into the Intestine.

It was shown by Bock and Hoffmann[1] that the injection of 1% NaCl solution into the blood of a rabbit caused a transient glycosuria. Large quantities of the solution were injected at the rate of 25–30 c.c. in 5 minutes, and the glycosuria appeared in from 20 minutes to 1 hour 30 minutes. After a number of hours (6–7) the glycosuria diminished in their experiment and finally disappeared, although the infusion and the flow of urine continued. They found the entire quantity of sugar secreted was in one case 1.632 g. and in another case 2.04 g. The percentage of sugar in the urine reached 0.136 and 0.219 respectively. These facts were confirmed by Kulz,[2] who found also that section of the splanchnics prevented this glycosuria. The experiments lately published by M. H. Fischer[3] show that glycosuria is produced by certain of the salts which were proven by Loeb[4] to produce muscular twitchings, and that calcium, which was shown by Loeb to suppress these twitchings, has also the power of suppressing the glycosuria.

From the fact shown above that the secretion of fluid is increased in the intestine when the kidneys are tied off and salt solution injected into the blood, I was led to inquire whether the intestine also secretes sugar in the absence of the kidneys under conditions which ordinarily bring about glycosuria. A number of experiments were made to determine this. I have found, in

[1] Bock and Hoffmann: Arch. für Anat., Physiol. und wissenschaftl. Med. (Reichert und DuBois-Reymond), p. 550, 1871.

[2] Külz: C, Eckhard's Beiträge, Bd. 6, S 117, 1872. (Quoted by Pflüger, Arch. für die gesammte Physiologie, Bd. 96, 1903, S 313.)

[3] M. H. Fischer: University of California Publications, Physiology, Vol. I, pp. 77 and 87, 1904.

[4] J. Loeb: Festschrift für Fick, 1899; Pflüger's Archiv., 1902, XCI, p. 248.

short, that when $\frac{m}{6}$ NaCl solution is injected into the blood in large quantities sugar is abundantly secreted into the intestine. It was further found that this takes place to a certain extent, also, when the kidneys are intact and are also secreting sugar. This may be seen in the following experiments.

Experiment 5.

Rabbit.—Blood vessels of kidney ligatured; cannula in upper part of small intestine with loop 35 cm. long tied off. Loop emptied; contents 5 c.c. which contained no sugar.

Time.	NaCl $\frac{m}{6}$ injected.	Intestinal juice.	Sugar examination of intestinal juice.
10:00	Infusion begun	Loop emptied, 5 c.c.	No sugar.
10:30	20 c.c.	3 c.c.	No sugar.
11:00	40 "	3 "	No sugar.
11:30	20 "	4 "	No sugar.
12:00	120 "	6 "	Trace of sugar.
12:30	200 "	12 "	Sugar abundant.
1:00	— "	28 "	Sugar abundant.
	400 "		

Experiment 6.

Rabbit.—Operation similar to preceding. Loop 30 cm. long contained 3 c.c. fluid which showed no sugar reaction.

Time.	NaCl $\frac{m}{6}$ injected.	Intestinal juice.	Sugar examination of intestinal juice.
10:00	Infusion begun.	Loop emptied, 3 c.c.	No sugar.
11:00	30 c.c.	7 c.c.	No sugar.
12:00	120 "	7 "	Considerable trace of sugar.
12:30	30 "	6 "	Sugar abundant.
	180 "		

In the latter experiment the sugar appeared after a relatively small amount of fluid had been injected; but in both cases it is seen that the *intestinal glycosuria*, if such name may be applied to this phenomenon, appears under circumstances which are exactly the same as those necessary to the production of sugar in the urine by saline infusions. A further example of the same phenomenon is shown in the following experiment:

Experiment 7.

Rabbit.—Blood vessels of both kidneys ligatured. Intestinal loop including duodenum 32 cm. long. In the loop were found 3 c.c. fluid which contained no sugar.

Time.	NaCl $\frac{m}{6}$ injected.	Intestinal juice.	Sugar examination of intestinal juice.
9:45	Infusion begun.	Loop emptied, 3 c.c.	No sugar.
10:15	10 c.c.	3 c.c.	No sugar.
10:45	70 "	3 "	No sugar.
11:15	75 "	3.2"	No sugar.
11:45	85 "	5 "	Sugar abundant { 0.202%. 0.222%.
12:15	80 "	9.5"	Sugar abundant 0.25%.
12:45	150 "	21 "	Sugar abundant.
	470 "		

The infusion was stopped at this point and the animal killed. The remaining loops of small intestine contained 32 c.c. fluid which contained sugar. The stomach contained about 40 cc. of fluid which also contained sugar. This will be spoken of again. Of the 470 c.c. of fluid injected 78.7 c.c. were eliminated by the small intestine and 40 by the stomach. The alimentary canal, then, exclusive of the large intestine, eliminated about 118 c.c. of the fluid introduced, which is approximately 25%.

Quantitative estimates were made of the sugar contained in the intestinal juice in this experiment. The percentage was found to be very considerable, varying between 0.2 and 0.3%. I have not attempted to ascertain the total quantity of sugar which may be obtained from the intestinal juice by continued infusion of salt solution. Such estimations in the case of urine were made by Bock and Hoffman, who found that the kidneys eliminated in one case 1.632 g. and in another case 2.04 g. sugar. The percentage concentrations in these cases were 0.136 and 0.219% respectively. M. H. Fischer found that the concentration of sugar in the urine after infusion of $\frac{m}{6}$ NaCl rarely exceeds 0.25%.

It is thus seen that the intestine excretes sugar in a way that entirely resembles its secretion by the kidneys. It seems that the infusion of $\frac{m}{6}$ NaCl solution causes sugar to appear in the blood. On reaching a certain concentration it is secreted by the kidneys. If the kidneys are cut off from the circulation the sugar is excreted by the intestine. Accompanying this intestinal gly-

cosuria there is a condition brought about in the intestine which is analogous to polyuria. In other words, in the absence of the kidneys the infusion of $\frac{m}{6}$ NaCl solution produces a largely increased secretion of intestinal fluid (intestinal polyuria) and at the same time a secretion of sugar into the intestine (intestinal glycosuria). That these conditions, however, are not entirely dependent on the absence of the kidneys is shown in the following experiment, where the kidneys are kept intact. *Not only do true polyuria and glycosuria occur where the intestine is intact, but also it is possible to produce a greater secretion of fluid and a secretion of sugar into the intestine when the kidneys are actively functioning.*

Experiment 8.

Rabbit.—Cannula placed in bladder. Cannula in loop of upper part of small intestine 35 cm. long. Loop contained 4.2 c.c. fluid—no sugar.

Time.	NaCl $\frac{m}{6}$ injected.	Intestinal juice.		Urine.	
		Quantity.	Sugar examination.	Quantity.	Sugar examination.
9:45	Infusion begun.	Loop emptied, 4.2 c.c.	No sugar.	Bladder emptied, 5 c.c.	No sugar.
10:15	5 c.c.	1.8 c.c.	" "	0.0	
10:45	50 "	2.2 "	" "	0.0	
11:15	80 "	2.9 "	" "	4.0 c.c.	No sugar.
11:45	92 "	3.8 "	" "	15.0	Sugar present.
12:15	120 "	6.6 "	Sugar present.	38.0	Much sugar.
12:45	150 "	7.8 "	" "	40.0	" "
	497 "				

As shown in this experiment, the sugar appears both in the intestinal juice and in the urine. The quantity of sugar also is much greater in the urine than in the intestinal juice. Rough estimations with measured quantities of copper sulphate show that the sugar of the urine is about 0.2%. That of the intestinal juice is considerably less. The quantity of urine secreted is, however, much greater than the quantity of intestinal juice. The greater proportion of the sugar is therefore secreted by the kidneys.

It is impossible to compare the results obtained in the different animals in one of which the kidneys have been tied and in the other left intact. In general, however, it may be stated that

in those animals in which the kidneys are functioning less fluid and less sugar are secreted by the intestines under the same circumstances and in the same time than is the case where the kidneys are removed from the circulation. This is *a priori* to be expected, since, if the rate of infusion be constant, neither the volume of the blood, nor its concentration in NaCl, nor the percentage of sugar it contains, is so great when the kidneys are actively secreting as when they are removed.

In another article[1] I have shown that the blood pressure is not essential for the secretion of fluid into the intestine, since loops of intestines removed from the body can be made to secrete fluid by immersion in various saline solutions. The blood supply seems to be important only in bringing oxygen to the tissues and in affording a constant supply of fluid to replace that secreted by the glandular tissues. The quantity of fluid secreted seems to bear a distinct relation to the quantity present in the tissues. The stimulus for secretion, however, in the case of infusions of NaCl solutions depends on the concentration of NaCl or its ions, presumably the Na ions. When, therefore, the kidneys are removed and no fluid is being eliminated in this way from the body, it seems certain that with a constant rate of infusion not only the quantity of fluid in the tissues, but also the concentration of the NaCl, must be increased. The conditions must, therefore, be more favorable for the secretion of large quantities of fluid by the intestine than is the case in the presence of the kidneys. The same reasoning naturally applies to the secretion of sugar by the intestine. Since its production as far as we know remains constant, its concentration in the blood must rise more rapidly in the absence of the kidneys than when they are actively eliminating sugar.

III.—The Secretion of Sugar by the Stomach.

As shown in experiment 7, a much increased flow of gastric juice was caused by the infusion of $\frac{m}{6}$ NaCl solution. In the absence of the kidneys in this case about 90 c.c. of fluid were eliminated by the stomach in 3 hours, 470 c.c. NaCl solution having been injected. In a second instance 390 c.c. $\frac{m}{6}$ NaCl solu-

[1] University of California Publications, Physiology, Vol. I, p. 115, 1904.

tion were injected into the blood and 32.8 c.c. of fluid collected from the stomach in 2 hours and 30 minutes. By simply inserting a cannula in a rabbit's stomach little or no fluid can be obtained when there is no salt solution being injected. After the saline infusion the fluid obtained from the stomach is strongly acid; while the intestinal juice is slightly alkaline, or sometimes neutral. These reactions continue to be constant even when large quantities of salt solution are injected. This fact adds weight to the idea that the elimination of large quantities of salt solution introduced into the blood is not merely a filtration process, but a process of active secretion. The study of the acidity of the gastric juice under these circumstances is being continued.

It was found on examination that the fluid secreted into the stomach after infusion of $\frac{m}{6}$ *NaCl solution also contained sugar.* The normal gastric juice was found to contain no sugar. This substance appeared in the fluid secreted only after the injection of a considerable quantity of $\frac{m}{6}$ NaCl solution, *i.e.*, under circumstances similar to those under which it is secreted by the intestine and kidneys.

In this connection it is interesting to note that Claude Bernard[1] describes the presence of sugar in the gastric contents of diabetic patients. He quotes MacGregor as having made the observation by causing diabetic patients to vomit. On examining the stomach contents sugar was demonstrated. It seems possible in this case that the food might have contained a reducing substance.

IV.—The Secretion of the Urea Into the Alimentary Canal.

The conception of the intestine as a supplementary excretory organ has been mentioned above; and the analogy which exists between the secretion of the kidney and that of the intestine has been pointed out. This idea is further strongly supported by the fact that urea is normally excreted by the intestine—and, indeed, also by the stomach, the salivary glands and liver. Claude Bernard[2] states that urea is to be found in the saliva and

[1] Leçons sur les propriétés physiologiques des liquides de l'organisme. T. II. 1859, p. 74.

[2] Leçons sur les propriétés physiologiques et les altérations pathologiques des liquides de l'organisme. II Tome Paris, 1859. Deuxième Leçon.

in the gastric juice; that in nephritis and after removal of the kidneys urea is excreted by other organs, more especially by the intestine. In these cases urea is usually found in the intestine in very minute quantities and sometimes not at all. The intestinal juice, however, is found to contain large quantities of ammonia salts. These are according to Claude Bernard decomposition products of the urea which is excreted into the intestine. Urea introduced into the intestine was found to be quickly decomposed with similar products. Claude Bernard thus concludes that after nephrotomy the intestine supplements the kidneys in eliminating the materials of the urine. Pregl[1] demonstrated the presence of urea in the intestinal juice of the sheep. He found that its concentration was 0.248%. In the intestinal juice of rabbits I have found that small quantities of urea exist both before and after the extirpation of the kidneys. It is present not only in the normal intestinal juice, but also in the fluid obtained from the intestine after the infusion of large quantities of $\frac{m}{6}$ NaCl solution.

In this connection it is interesting to note the statement made by Weintrand[2] that uric acid is excreted by the intestine.

All of these facts which have been described above, namely the intestinal polyuria, the intestinal glycosuria, the excretion of sugar into the stomach, the excretion of urea by stomach, intestine, salivary glands and liver, and the excretion of uric acid by the intestine, strongly support the idea that the intestines and, perhaps also other parts of the alimentary canal have a distinct excretory function which is supplementary to that of the kidneys. When the kidneys are absent the intestines can, to a certain extent, take on their functions; and when the excretion of urea, etc., by the urine is lessened by disease of the kidneys, notably in nephritis, it is rational to increase the secretion of the intestinal juice by the administration of purgatives, a procedure which which has long been empirically employed.

This function of the intestine, however, is not a truly vicarious secretion since no substances are eliminated by the intestine when the kidneys are absent which may not also be excreted in smaller quantity by the intestine when the kidneys are active.

[1] Archiv. für die gesammte Physiologie. Bd. 61, 1895, p. 378.
[2] Chemische Centralblatt. Leipzig Bd. II, 1895, S. 310.

V.—Conclusions.

1. The intravenous injection of large quantities of $\frac{m}{8}$—$\frac{m}{6}$ NaCl solution causes a greatly increased secretion of fluid into the intestine, which is the more marked, the more rapid the injection.

2. This increased secretion of fluid into the intestine is analogous to the production by the same means of polyuria. The action of the salt solution on the intestine is the same as that of the saline purgatives, and is analogous to diuresis.

3. The intravenous injection of large quantities of $\frac{m}{6}$ NaCl solution causes marked excretion of sugar by the intestine. The concentration of this substance in the intestinal juice may be as high as 0.25%.

4. This excretion of sugar by the intestine is analogous to the excretion of sugar by the kidneys (glycosuria) produced by the same means.

5. Under similar circumstances sugar is also excreted by the stomach.

6. Urea is found in the intestinal juice normally in small quantities, and also after saline infusions.

7. All of these facts support the idea that the intestine is in part an organ of excretion supplementary to the kidney. The secretory activities of the two organs are stimulated in many cases by the same salts (NaCl, Na_2SO_4, $BaCl_2$, sodium citrate, etc.,) and are suppressed by calcium. Sugar and urea are excreted by the intestine under the same circumstances which accompany their secretion by the kidneys.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 15, pp. 139-147 April 5, 1904

ON THE INFLUENCE OF THE REACTION OF THE SEA-WATER ON THE REGENERATION AND GROWTH OF TUBULARIANS.[1]

BY

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Five years ago I reported experiments on the influence of acids and alkalies on the development of the larvæ of sea urchins. I found that the addition of acids to the sea-water delayed the development, and finally, at a certain concentration of hydrogen ions, inhibited the development completely. With the addition of alkalies the development during the first twelve hours was not at all hastened, or hastened to a hardly appreciable extent. On the second day, however, and sometimes also even on the third day, the eggs that developed in sea-water which had been made alkaline were, occasionally but not always, with reference to the rapidity of the development and growth in advance of eggs, of the same brood which had been raised in normal sea-water.[2] Since at that time it was generally accepted that the sea-water, as well as the blood, had an alkaline reaction, this observation seemed to indicate that for developmental processes it is necessary to have an alkaline reaction, or, in other words, a higher concentration of hydroxyl ions than exists

[1] Translated from Pflüger's Archiv., Vol. 101, p. 340, 1904, by John Bruce MacCallum.

[2] Loeb: Archiv. f. Entwickelungsmechanik, Bd. 7, S. 631, 1898.

in distilled water. After it had been demonstrated, however, by Friedenthal, Fraenkel, and Farkas that this assumption is not true for blood, and I myself have shown that sea-water has a practically neutral reaction,[1] a new investigation of the significance of the reaction of sea-water for the processes of development and growth was made necessary.

I have studied the effect of the reaction of the sea-water upon the regeneration of the amputated polyps in Tubularia crocea (a hydroid) and the growth or increase in length of the stem following this regeneration.[2] Since it was desirable to work with artificial solutions instead of sea-water (which itself contains carbonates and phosphates), it was first determined in what solutions the regeneration of the amputated polyps is possible.

The solutions were prepared with the purest salts of commerce (Kahlbaum) and the distilled water was investigated as to its electrical conductivity before it was used. The concentration of the salt solutions employed was $\frac{3}{8}$ grammolecular, which represents approximately the concentration of the sea-water in the Bay of San Francisco, where the hydroids used in the experiments were obtained. Preliminary experiments showed that the regeneration of the amputated polyps of Tubularia was within wide limits independent of the concentration of the sea-water. Even in a mixture of equal parts of sea-water and distilled water the regeneration occurred, although with a slight diminution of velocity. In a mixture of 40 parts of sea-water with 60 parts of distilled water regeneration usually no longer occurred. If for the dilution of the sea-water a $\frac{3}{8}$ grammolecular or weaker sugar solution was used instead of distilled water, a slight dilution of sea-water sufficed to prevent regeneration. In the best case a mixture of 70 parts of sea-water with 30 parts of the sugar solution was the utmost limit of dilution with sugar solution at which regeneration was still observed. As a rule in a mixture of 90 parts of sea-water and 10 parts of sugar solution the poisonous action of the sugar manifested itself in the fact that the animals soon died, and only a few formed new polyps.

[1] Loeb: Archiv. für die gesammte Physiologie, Bd. 99, 637, 1903.

[2] Details concerning regeneration and growth in Tubularians have been published by me in my two monographs, "Untersuchungen zur physiologischen Morphologie der Thiere," I and II (Würzburg, 1890 and 1891).

These results were obtained with dextrose, cane sugar, and lactose. Chemically pure sugar was always used, and when it was found that the sugar solutions were so much more poisonous than distilled water, the sugars were recrystallized before they were used. The solutions were always used only when they were quite fresh, in order to avoid the possibility of the transformation of the sugar by organisms of fermentation. I presume that the sugar enters the cells of these animals and here gives rise to the formation of injurious compounds.

In an earlier research I have shown that the toxicity of distilled water for most marine animals certainly depends not in general and not exclusively on the difference in the total osmotic pressure; but that many marine animals die in distilled water because the distilled water lacks certain salts, namely the salts of Na, Ca, and K.[1] As a fourth metal Mg must be considered, but not with the same degree of necessity. As far as the anions are concerned Cl is sufficient. While several different kations are necessary for the maintenance of life, this does not seem to be true to the same extent with regard to the anions. It is possible that this difference is connected in some way with the observations which I have previously published on the toxic and antitoxic actions of ions.[2]

Experiments which I made on the regeneration of the polyps of Tubularians showed that regeneration of the polyps occurs in artificial mixtures of solutions of NaCl, KCl, $CaCl_2$, and $MgCl_2$, which contain these constituents in about the same proportion as they occur in the sea-water. The addition of $MgSO_4$ improves the result only slightly. Individual constituents such as $CaCl_2$ may undergo considerable variation in concentration without rendering the regeneration impossible. These facts are on the whole a confirmation of the experiments which I made on the same subject in Naples and have published in the "Untersuchungen zur physiologischen Morphologie der Thiere."[3]

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[1] Loeb: Archiv. f. die gesammte Physiologie, Bd. 97, S. 394, 1903.

[2] Loeb: Archiv. f. die gesammte Physiologie, Bd. 88, S. 68, 1901. Americ. Journal of Physiology, Vol. 6. p. 411. 1902. Loeb and Gies: Arch. f. die gesammte Physiologie, Bd. 93, S. 246, 1902.

[3] In these experiments instead of distilled water fresh water was used which contained a slight amount of Ca salts.

We come now to the new experiments on the significance of the reaction of the solution for regeneration and growth. The starting point for these experiments was the observation that in a mixture of solutions of NaCl, KCl, $CaCl_2$, and $MgCl_2$ with or without $MgSO_4$ the amputated polyps will regenerate, but that the increase in the length of the stem following the regeneration in sea-water is entirely or almost entirely absent in the mixtures mentioned above. Since sea-water is in general not an alkaline solution, it became necessary to determine the cause of this behavior. There existed a second difference between the behavior of the regenerating stem in sea-water and in the solutions mentioned. In the former, regeneration of the amputated polyps took place at the room temperature prevailing during summer (about 20° C) in about two days, while in a mixture of the solutions of NaCl, KCl, $CaCl_2$, and $MgCl_2$ the process occupied about three days.

The new experiments showed that the addition of NaHO, $NaHCO_3$, and of Na_2HPO_4 to this artificial mixture rendered the rapidity of regeneration and growth in this solution almost or fully equal to that in sea-water.

A couple of examples may illustrate this. Van't Hoff's solution served as the artificial solution. This consisted of

100 NaCl , 2.2 KCl , 1.$CaCl_2$, 7.8 $MgCl_2$, 3.8 $MgSO_4$

in $\frac{3}{8}$ grammolecular concentration. Each 100 c.c. of this solution were placed in a flat vessel which was covered loosely with a glass plate. Then varying quantities of NaHO and Na_2CO_3 were added to these solutions. In each vessel 8 pieces about 5 cm. long taken from the Tubularian stem were placed. All the stems came from the same colony, and for every experiment stems were used which came fresh from the ocean. This last is an essential condition, since the animals live in dense colonies and contend with one another for the oxygen. In this condition they soon die from lack of oxygen when kept in small dishes. If, on the contrary, 6 to 12 stems be isolated and placed in separate vessels, they remain living.[1] The following solutions were employed:

[1] Such stems are still alive to-day, *i.e.*, after seven to eight months.

(1) 100 c.c. Van't Hoff's solution.

(2) 100 " " " " + 0.1 c.c. $\frac{n}{10}$ NaHO

(3) 100 " " " " + 0.2 " $\frac{n}{10}$ "

(4) 100 " " " " + 0.3 " $\frac{n}{10}$ "

(5) 100 " " " " + 0.4 " $\frac{n}{10}$ "

(6) 100 " " " " + 0.5 " $\frac{n}{10}$ "

(7) 100 " " " " + 0.6 " $\frac{n}{10}$ "

(8) 100 " " " " + 0.7 " $\frac{n}{10}$ "

(9) 100 " " " " + 0.8 " $\frac{n}{10}$ "

(10) 100 " " " " + 0.9 " $\frac{n}{10}$ "

(11) 100 " " " " + 0.05 c.c. $\frac{5}{8}$ m. Na_2CO_3

(12) 100 " " " " + 0.1 " $\frac{5}{8}$ m. "

(13) 100 " " " " + 0.15 " $\frac{5}{8}$ m. "

(14) 100 " " " " + 0.2 " $\frac{5}{8}$ m. "

(15) 100 c.c. normal sea-water.

In solutions 1, 2, and 3 regeneration of the polyps does not take place quite so rapidly as in sea-water; on the whole the regenerative processes remain about 24 hours behind those in sea-water. In the other solutions, however, regeneration occurs just as rapidly as in sea-water.

After six days the growth in the length of the individual stems was measured in all these solutions. The details of the method of this measurement are to be found in my earlier publications. In solutions 1 to 4 the growth in length was almost nil; in the solutions 5 to 12 on the contrary it was very well marked, and in the best cases as great as in the control material left in sea-water. For the stems in solution 5 it amounted on an average to 4½ mm., in solution 7, 9 mm., in solution 9, 6¼ mm. In solutions 13 and 14 the polyps soon fell off from the stem. The concentration of the Na_2CO_3 was evidently too high.

These experiments (which were repeated four times) made it appear as if the free hydroxyl ions were necessary for the growth of the stem, and still this was incompatible with the fact that the reaction of sea-water is neutral.

Experiments were therefore made in which to the van't Hoff solution was added a solution of $NaHCO_3$ (through which a stream of CO_2 had been passed for an hour before the beginning of the experiment) and Na_2HPO_4. (The addition of this material did not render the van't Hoff solution alkaline). It proved that the action of $NaHCO_3$ to the van't Hoff solution was still more favorable than that of NaHO. If 0.5 to 1.0 c.c. (or even more) of a $\frac{3}{8}$ grammolecular solution of $NaHCO_3$ were added to 100 c.c. of the van't Hoff solution the regeneration and growth were accelerated considerably, and attained the rapidity with which these processes proceed in the control material in normal sea-water. Na_2HPO_4 acted similarly to $NaHCO_3$. Sulphates and citrates, however, did not produce any favorable effect.

These experiments which were repeated and modified very often, prove that the favorable action of sodium hydrate depend not on a direct accelerating action of the hydroxyl ions on the growth, but on a property which is common to NaOH and $NaHCO_3$, and Na_2HPO_4, and this is obviously the neutralization of an acid, which might be formed by the Tubularian stem (or by its parasites.)

That acids delay the regeneration and growth of Tubularia or render it impossible may be easily demonstrated. Addition of 0.05 c.c. and still more plainly 0.1 c.c. of a $\frac{n}{10}$ HCl solution to 100 c.c. of the van't Hoff solution markedly hinders the regeneration and growth of Tubularia, and an addition of 0.15 c.c. $\frac{n}{10}$ HCl to 100 c.c. of the neutral van't Hoff solution renders growth and regeneration impossible.

One of the above mentioned experiments on the action of NaOH requires an explanation. The addition of 0.8 and even 1.0 c.c. $\frac{n}{10}$ NaHO to 100 c,c. of van't Hoff's solution allowed regeneration to take place, while from earlier observations on muscle, on eggs and spermatozoa I expected that such a concentration would be injurious. Experiments with phenolphthalein showed that van't Hoff's solution, if NaOH is added to it, loses its alkalinity gradually, and the more rapidly the smaller the amount of sodium hydrate added. Neutralization evidently results from the absorption of CO_2 from the air. If 0.1 c.c. $\frac{n}{10}$ NaHO or 0.2 cc.. $\frac{n}{10}$ NaHO were added to 100 c.c. of van't

Hoff's solution, phenolphthalein produced after 2 or 3 hours no longer any red coloration. With the addition of 0.8 c.c. $\frac{n}{10}$ NaHO to 100 c.c. of van't Hoff's solution, about 18 hours elapsed before the red coloration disappeared in the dishes used for the experiments. We thus see that even a relatively considerable addition of sodium hydrate did not produce a permanently injurious effect, because the greater part of the free hydroxyl ions were eliminated by the carbonic acid in the course of the first 24 hours.

This observation further explains the fact which otherwise would not be intelligible, namely, that addition of 0.1 to 0.3 ccm. $\frac{n}{10}$ NaHO to 100 ccm. of van't Hoff's solution influenced the rapidity of regeneration and growth little, or not at all. The reason clearly is: because the alkali is neutralized after a few hours by the carbonic acid absorbed from the air, while the process of regeneration requires at least two days.

This observation possibly explains also why in my older experiments on sea-urchin's eggs, scarcely any accelerating action on the growth was observed during the first 12 hours, caused by the sodium hydrate added to the sea-water, while somewhat later this action was, in a number of cases at least, very marked. The concentration of the hydroxyl ions was in the beginning probably too high, and only after the absorption of a sufficient quantity of carbonic acid was the proper concentration of hydroxyl ions for the development reached. The conditions of equilibrium are, however, somewhat complicated by the addition of sodium hydrate to sea-water, and I shall therefore not express too definite an opinion on this point.

After I had convinced myself of the beneficial action of the addition to the van't Hoff solution of such substances as are capable of neutralizating strongly dissociable acids, I enquired whether a solution of three chlorides would not suffice for the regeneration of Tubularia, provided that a neutralizing agent was added. I found that regeneration took place in a solution of NaCl KCl, and $CaCl_2$ if only some $NaHCO_3$ were added. $NaHCO_3$ is in this respect more favorable than NaHO, because it suffices for the neutralization of somewhat stronger acids without exerting the injurious effects which hydroxyl ions exert when

their concentration is too high. With the employment of alkali in the case of slowly progressing life phenomena one has always to choose between two evils which are dependent on the rather rapid neutralization of the alkali by the carbonic acid from the air or the animals; one must either add the alkali in the beginning in an excessive concentration and thus in the beginning injure the cells, or the alkali becomes neutralized before it has time to act beneficially. The only method by which the evil might be avoided consists in the repeated addition of small quantities of sodium hydrate. I have not yet tried this method since the same object could be attained more readily by the use of $NaHCO_3$.

It has been known for a long time that animals live better in aquaria when the latter contain green plants and are exposed to the light. This has been correctly attributed to the rapid removal of the carbonic acid formed by the animals, by the assimilating action of the green plants. The foregoing observations point toward the possibility that in this process still another function belongs to the green plants. Since sea-water contains bicarbonate the addition of algae must, if the sea-water is exposed to light, hasten the transformation of the bicarbonate into carbonate and thus increase the concentration of the hydroxyl ions. When I tested with phenolphthalein the reaction of sea-water which contained green algæ (Ulva) and had been exposed to diffused daylight for many hours, a bright red coloration resulted. The red color was absent if the sea-water with the algæ remained in a dark room. I found, however, further, that the algæ in the light rendered a van't Hoff's solution alkaline which contained no carbonate.[1] This almost gave the impression that one was dealing with a direct excretion of an alkali by the algæ under the influence of light. One might from the analogy of certain land plants think of the secretion of calcium carbonate. This subject will be investigated further.

Experiments on other hydroids, e.g. Obelia, gave results similar to those recorded in this paper on Tubularia.

[1] These observations were made in the course of experiments which I made for another purpose together with my colleague, Professor Osterhout.

We see thus that in a neutral mixture of solutions of NaCl, KCl, $CaCl_2$ and $MgCl_2$ in the proportion in which these salts are contained in sea-water, the regeneration of amputated polyps of Tubularia takes place; that the growth, however, occurs much more slowly in such a mixture than in sea-water. If one adds to a mixture of solutions of NaCl, KCl, $CaCl_2$ and $MgCl_2$ however, a small but definite quantity of $NaHCO_3$ or Na_2HPO_4 one may considerably accelerate the growth, and make it even equal to that in normal sea-water. Since the addition of a small quantity of sodium hydrate acts similarly, though not quite so favorably, one gains the impression that in the Tubularian stem an acid is formed which delays the growth if it is not neutralized. Certain marine algæ under the influence of light seem to secrete substances which give to neutral solutions an alkaline reaction. These algæ may help to keep the reaction of the sea-water approximately neutral.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 16, pp. 149–150 May 21, 1904

THE POSSIBLE INFLUENCE OF THE AMPHOTERIC REACTION OF CERTAIN COLLOIDS UPON THE SIGN OF THEIR ELECTRICAL CHARGE IN THE PRESENCE OF ACID AND ALKALIS.

BY

JACQUES LOEB.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

Through the papers of Freundlich and Billitzer attention has been called to the fact that the sign of the electrical charges of the particles in a colloidal solution harmonizes with the idea that these charges are due to electrolytic dissociation. Colloids which are alkaline are positively charged and colloids that are acid are negatively charged. In the case of the acid colloids hydrogen ions and in the case of alkaline colloids hydroxyl ions are given off leaving the particles themselves negatively and positively charged respectively.

There is one group of facts which does not seem to fall under the same general conception, namely, Hardy's observation that dialyzed white of egg assumes a positive charge in a watery solution when a trace of acid is added to the solution and a negative charge when a trace of alkali is added.[1] Later on Hardy found that globulines behave in the same way under the influence of acids and alkalis.[2] Hardy's view of the influence of acids and alkalis on the sign of the charge of the colloidal particle is as

[1] W. D. Hardy; Journal of Physiology, Vol. 24, p. 288. 1899.

[2] Journal of Physiology, Vol. 29. 1903.

follows: "The particles of gel when suspended in a fluid containing ions are penetrated by those ions. Let the fundamental assumption be that the higher the specific velocity of an ion the more readily will it become entangled with the colloid particle. Then as H and HO ions have by far the highest specific velocity the colloid particle will entangle an excess of H ions in acid and thereby acquire a + charge and of HO ions in alkali and thereby acquire a — charge."[1]

It seems to the writer, however, that a different view of these phenomena is possible whereby they appear in harmony with the view of the electrolytic origin of the charges of colloids. The proteids are known to be amphoteric in their reaction. If they be slightly dissociable they will send H as well as HO ions into the solution. When the particles send more H ions than HO ions into the solution they will have a negative charge while they will have a positive charge when more HO ions are given off than H ions. If acid is added to the solution in sufficient concentration the amphoteric colloidal particle will send more HO ions into the solution than H ions and hence will assume a positive charge. The reverse will be the case in an alkaline solution. It harmonizes with this idea that, as Hardy found, neutral salts do not influence the sign of the electrical charge of the globulines.

[1] Hardy; Journal of Physiology, Vol. 29. 1903.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 17, pp. 151-161. May 21, 1904

CONCERNING DYNAMIC CONDITIONS WHICH CONTRIBUTE TOWARD THE DETERMINATION OF THE MORPHOLOGICAL POLARITY OF ORGANISMS.[1]

(First Communication.)

BY

JACQUES LOEB.

(With seven figures in the text.)

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

1. In the years 1890 and 1891 I published two monographs on the "Physiological Morphology of Animals" in which was reported among others a series of experiments on the nature of the conditions which determine the morphological polarity of the animal body. By morphological polarity I mean the fact that a piece cut off from the organism at that end which in the uninjured animal was directed towards the oral pole, again forms an oral pole, while at the opposite cut end an aboral pole is formed. If one cuts for example a square piece from the wall of Cerianthus, an Actinian, whose body is comparable with a hollow cylinder, and if one side of the square is parallel to the long axis of the animal, new tentacles arise only at that side of the square which was directed towards the former oral pole of the animal. What is the nature of the conditions which determine this kind of polarity? Some authors following the example of Vöchting assume that the individual cells are polarised and therefore produce at the basal end organs different from those at the oral end. My experiments gave evidence opposed to this idea since

[1] Translated from Pflüger's Archiv, Vol. 102, p. 152, by J. B. MacCallum.

I succeeded under certain conditions in producing oral or apical organs also at the aboral or basal end of a fragment. This phenomenon which I termed heteromorphosis, is opposed to the idea that the morphological polarity of the organism is dependent upon a corresponding morphological polarity of the individual cells.

Du Hamel and Bonnet in the 18th century had developed ideas concerning the formation of organs which Sachs in his treatise on "Stoff und Form der Pflanzenorgane"[1] again took up. According to du Hamel certain sap currents are responsible for the fact that in plants at one end of a piece cut off from a branch an apex arises and at the opposite end a root is formed. He assumed that a specific sap causes the formation of roots and that this sap moves basalwards in the stem of the plant, while the fluids which give rise to the formation of branches move apicalwards. Sachs generalised this idea and supposed that as many specific materials, or at least differences in the materials, —which are possibly only of a quantitative nature—exist in plants as there are different organs present in the plant. He showed that the facts of regeneration fit in with this conception. If a piece be cut from a branch of willow for example, roots form at the basal end of the fragment, and branches at the apical end. Sachs asks why the ends which would never have formed new organs without the injury suddenly begin to regenerate after the section. His answer is: because the root-forming materials which would have flowed into the roots before the section must now collect at the basal cut-end of the stem and thereby give rise to the conditions necessary for the growing out of the root germs present there; while the branch-forming materials which otherwise would have flowed towards the ends of the branches now collect at the apical cut-end and afford the conditions for the sprouting of a branch. This idea that the sap currents and the collecting of materials, form the cause of regeneration and organ formation was explained by Sachs in so many different examples that for my part no doubt remained that here a fruitful scientific conception was produced. Applied to the regeneration of pieces of Cerianthus we should have to say that in the wall of

[1] Sachs; Gesammelte Abhandlungen, Bd 2, s. 1159. Leipzig 1893.

Cerianthus a current of materials takes place, in the sense that, materials which favor the formation of tentacles flow exclusively or in greater abundance towards the oral end of the cut-piece of Cerianthus.

In order to test this view I made experiments on another animal in which the migration of material which apparently if not certainly was associated with the formation of organs could be directly observed, namely, a hydroid, Tubularia mesembryanthemum. Such an animal consists of a long tubular unbranched stem *SS* (Fig. 1), a root *W* (aboral pole) usually

Fig. 1.

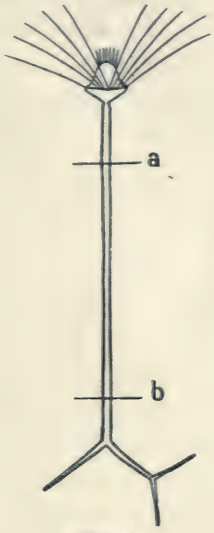


Fig. 2.

branched, and a polyp or head *P* (oral pole). The outer surface of the stem is surrounded by a hard (chitinous?) material, the periderm, and the living cells lie inside this tube. I found that if a piece *ab* (Fig. 2) be cut out from the body of the animal, *always* a new polyp and *never* a root is formed at the oral cut end *a*. At a sufficiently high temperature this takes place in two to three days. At the aboral cut end *b* of the segment there may be formed either a root or a polyp. If a polyp forms at the aboral end, it arises much more slowly often a week later than the polyp at the oral end. If anyone were to cut a piece *ab* from the stem of a Tubularian one could in general tell with certainty

from the phenomena of regeneration whether *a* or *b* were the oral cut end. For at the oral cut end the polyp generally forms earlier than at the aboral pole, provided a polyp and not a root is formed at the latter pole, or—what also occurs—no regeneration at all ensues at this end. A piece cut from the stem of the Tubularian therefore shows a decided polarity.

I found further that at that end at which a new polyp forms, the formation of this organ may be recognized beforehand from the fact that red pigment granules collect at this end in relatively great density. New pigment granules are transported by a current of fluid to this end, and evidently remain lying or stuck in the neighborhood of the cut surface. The stream of fluid is kept up by ciliary movement. I expressed at that time the opinion that such a transportation of materials by the stream of fluid towards the cut end is one of the conditions of the formation of organs in Tubularia, and that the polarity of the Tubularian stem, *i.e.*, the fact that the polyp forms earlier at the oral pole, depends on the fact that the "organ forming" substances (possibly the red pigment granules) collect in sufficient quantity sooner at the oral than at the aboral end. This assumption was based on a series of experiments, two of which I shall cite here. If a piece *ab* (Fig. 3) be cut from the stem of a Tubularian and

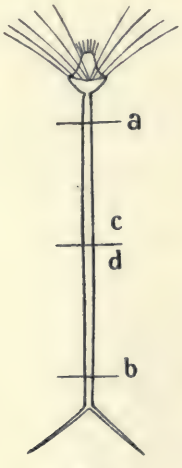


Fig. 3.

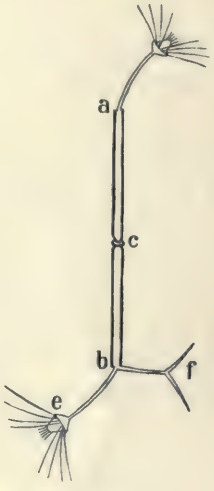


Fig. 4.

this piece be divided by a section passing between *c* and *d*, roots are never formed at the ends *a* and *d*, but always polyps; and the polyp formation usually begins equally quickly at *a* and *d*. At the aboral ends *c* and *d*, on the contrary, roots are formed; or if polyps are formed their formation begins later than at the ends *a* and *d*. The same results are obtained if the stem of a Tubularian be divided into four or eight instead of two pieces.

The second experiment which I wish to call attention to here it is better to quote literally: "If the Tubularian stems, which are subjected to the experiment, are very turgescent and provided with much pigment, the period of time existing between the formation of the oral and aboral polyps may be relatively small, and amount to only a few days. Very often, however, one finds colonies of pale looking Tubularians which form polyps at the oral pole in the normal time, in which, however, the aboral polyps arise not until one or several weeks later. With such animals I made the following experiment. I placed eight animals (I) of a colony vertically but reversed, *i.e.*, with the oral end in the sand in order to suppress the formation of polyps at this end. At the same time I fastened seven other animals (II) of the same colony likewise vertically and reversed—but so that both ends were surrounded by sea water. These animals were forced to form polyps at both cut ends. The animals of groups I and II were kept in the same aquarium and at the same temperature. After three days some new polyps first formed at the oral pole of the Tubularians (II) which were surrounded at both ends by sea water, and on the next day polyps had formed at *all the oral ends* of this group (II). On the same day polyps also formed on five animals of the other group (I) *at the aboral end;* and on the next day the regeneration was complete in all the animals of this group. In the division II on the contrary, where the animals had formed polyps at the oral pole, the first regeneration at the aboral end took place only five days later, and only after an additional six days was the polyp formation in this case complete. The formation of polyps at the *aboral pole* of those animals (I), in which the polyp formation was suppressed at the oral pole, was therefore completed about eleven days earlier than in the other animals of group II, in which the polyp formation occurred at the oral pole."

By inhibiting the polyp formation at the oral end one can, therefore, accelerate the polyp formation at the aboral end.[1] These investigations were repeated and confirmed by Driesch.[2]

2. If this phenomenon of polarity depends on a current, by means of which the polyp forming materials are carried away from the aboral cut end, or can not collect or form so quickly at this end, one should be able to do away with this polarity by inhibiting the current. This may be accomplished most simply by fastening a ligature around the middle of the stem. I have made such experiments during the last summer on a great number of stems of Tubularia chorocea, and have found that this expectation is in fact confirmed.

If one cuts a piece *a b* (Fig. 2) from the stem of a Tubularian and ties this in the middle at *c* by means of a thin silk thread, the formation of the aboral pole is hastened, and the polyps at *b* form usually almost as rapidly as the polyps at *a*. Further one observes that in all cases at the aboral end first a polyp arises and never a root.

Twenty-eight long stems of a Tubularian were chosen and the polyps and roots cut off. All the pieces were approximately equal in length. Fourteen of these pieces were tied in the middle by a fine silk thread. The remaining 14 served as a control. All the oral cut ends were oriented in the same way, and precautions were taken that the pieces could not be disturbed and no doubt could arise as to which was the oral and which the aboral end of any particular stem. On the second day all the ligated stems formed oral polyps and 13 (that is all but one) aboral polyps. The unligated control stems formed 13 oral polyps and *no polyps at the aboral end.* On the following day all 14 ligated stems formed polyps at the aboral end, while seven of the control stems formed polyps, and two stems roots, at the aboral end. The experiment may serve as a typical one *In the ligated stems at the aboral end, only polyps are formed and never an immediate root formation occurred, and the aboral polyps were formed approximately as quickly as the oral polyps.* The unligated stems on the contrary formed roots directly at the aboral cut end

[1] Loeb, Untersuchungen zur physiologischen Morphologie der Thiere. Bd. 2, 1891.

[2] Driesch, Archiv für Entwickelungsmechanik Bd. 5, 1897, and Bd. 9 S. 130, 1900.

in about 10–20 per cent of the cases, and the polyp formation at the aboral end without exception either occurred later than at the oral end, or did not occur at all. I tried then to determine whether it makes any essential difference where the ligature is placed on the stem, in the middle, near the oral cut end or near the aboral cut end. As a result I may mention that it is rather immaterial where the stem is ligated so long as the aboral piece lying behind the ligature is not too small. In this case the ligature may prevent the process of regeneration at this end. If we take this into consideration we may say that ligation of a piece cut from the stem of Tubularia results in the suspension of the polarity as far as regeneration is concerned. This holds good also if one cuts the stem into four or eight pieces and then ligatures each fragment in the middle or near the oral cut end as the following examples show.

The original Tubularian stems which were used for this experiment were about 8 cm. long or still longer. Twenty-four vigorous stems of the same colony were selected and each stem cut into four pieces of about the same size. The 48 fragments which were derived from 12 of the stems were all ligated near the oral cut end by a thin thread; the 48 fragments of the other 12 stems served as control material.

After 48 hours 36 of the 48 fragments which were ligated had formed a polyp at the aboral cut end, while only 6 of the 48 control pieces had formed an aboral polyp. On the next day 47 of the pieces with ligatures had formed an aboral polyp, while of the control pieces only 11 had formed an aboral polyp. On the following day the proportion was still the same. The oral polyps had formed in the majority as usual on the second day. The experiment shows strikingly that the ligation of the stem hastens the formation of the aboral polyps and brings it to almost the same order of rapidity as the formation of the oral polyps.

In another experiment 24 stems were cut each into 8 parts, and the 96 fragments of the first 12 stems were ligated near the oral end, while the remaining 96 pieces served as a control. After three days 75 of the 96 pieces with ligatures had formed an aboral polyp, while among those not ligated only 13 formed an aboral polyp. The majority had, however, formed an oral

polyp. The root formation was in these short fragments not as frequent as in the larger pieces of the previous experiment.

I mentioned above that 10–20% of the long pieces of stem formed a root at the aboral cut end; that, however, the stems with ligatures formed, practically without exception, a polyp at the aboral end (in many hundred cases). After some weeks I occasionally found that in some of these ligated stems a root had *subsequently* formed, and this root usually arose in the same place where the polyp had grown out. Figure 4 gives a schematic representation of this condition. *ab* was the piece of stem cut from the polyp; at *c* the stem was ligated. At the aboral end *b* a polyp *e* was formed, and the new stem segment *eb* arose by growth. After some weeks the new root *bf* grew out from *b*. This observation, however, was only seldom made. If we may judge this appearance on the basis of analogous processes in Antennularia and in plants we might assume that the root formation was caused by the new sprout *be*.

3. The experiments so far described had shown that by ligaturing a piece cut from the stem of the Tubularian one abolishes the difference between the oral and aboral poles. The question arises whether one can go farther and even reverse the polarity; *i.e.*, whether one can force the aboral cut end to form a polyp sooner than the oral end. This is in fact possible, though this phenomenon does not manifest itself with the same degree of certainty as the results of the ligature experiments just described.

Let *ab* (Fig. 5) be a piece of the stem of a Tubularian, *a* the

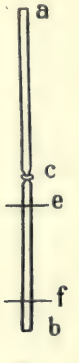


Fig. 5.

Fig. 6.

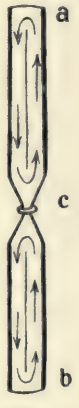


Fig. 7.

oral, *b* the aboral cut end. At *c* the piece of stem was ligatured immediately after it was cut. We wait until the new polyps have formed at *a* and *b*, which occurs about simultaneously, usually in about two days. On the following day a piece *ef* is cut out of the aboral piece *cb*. Will the new polyp form first at *e*, as would be expected if the old polarity still remained, or will new polyps form simultaneously at *e* and *f*, as would be expected if the action of the ligature still continued, or will the polyp form, at least in a portion of the cases, earlier at *f*? The last is actually under certain conditions the case, as the following example shows. Thirteen long stems of Tubularia were cut through in the middle and the aboral halves ligated near the anterior end with a silk thread. Only the latter pieces are of interest to us here. Three days after the operation the aboral polyps had formed in each stem at *b*, and now the portion *ef* was cut from the stem. Two days later 10 stems had formed an aboral polyp at *f* and only 5 stems showed an oral polyp at *e*. There then exists here a reversal of the normal polarity. Four series of experiments were carried out with similar results. In the repetition of this experiment, however, there are some points to be observed: In these experiments a piece *ef* was cut from the old stem. If a piece be cut so that the cut end *e* lies in the old stem while *f* lies in the newly grown piece, it is found that the result is often the opposite to that stated. This takes place especially, I believe, when the newly regenerated piece is poor in pigment and its circulation is not especially strong. It is also to be considered that if *e* lies too near the ligature *c* irregular results may be obtained, because, as I imagine, necrotic material is abundantly present in the place where the ligature is tied.

If we correlate all these observations, we get the idea that the cause of the normal polarity which appears in the regeneration of a piece cut from the stem of a Tubularian is based on the condition that the circulation favors the motion of various substances in the direction from the aboral to the oral pole.

4. The Tubularian stem is a long hollow cylinder, whose hollow space is divided lengthwise by a smooth membrane *m* (Fig. 6) into two separate halves. This membrane possesses at each end of the stem an opening through which the two halves com-

municate. If a piece be cut from the stem the openings at the ends *a* and *b* close in about an hour (at suitable temperature) and the membrane becomes broken through at the two ends *a* and *b*. By means of ciliary motion a circulation is established in the piece, which is directed on the one side towards *a* and on the other side towards *b*. In the circulating fluid pigment granules are found, red and yellow, which collect at the ends and perhaps remain stuck there. If *a* is the oral end they collect in greater abundance at *a*, and here also the first polyp is formed, while at the other end the polyp forms later or a root arises.

If one ligatures a piece such as *ab* (Fig. 7) in the middle at *c* there are formed at *d* new apertures in the partition wall *m*, and each portion has its own separate circulation.

It is now generally found that only those pieces form a new polyp which possess a circulation. It is further found that the collection of pigment granules at one cut end precedes the polyp formation. This connection may be made clear better if we split the stem lengthwise. It is then seen that the regeneration of a new polyp proceeds from those points where the pigment granules collect. It is further found that stems which are poor in pigment form polyps more slowly than stems rich in pigment. The pigment granules are carried with the circulation towards the cut end, where they gradually collect. Vortex movements and stickiness probably hold them there. They collect in stems which are split lengthwise in greater quantities only at the free cut ends, not in the inner parts of the stem. Likewise in ligation no collection of the pigment granules takes place at the places ligated, but only at the free ends, where the polyp is afterwards formed; and indeed usually this collection is most dense at that end where the polyp is first formed. (Perhaps the supply of oxygen is one of the conditions which directly or indirectly are connected with the sticking or collection of the pigment granules at the cut end.) All these facts together speak in favor of the idea that these granules, or a material which with regard to its accumulation acts in the same way as the pigment granules, forms the basis for the process of regeneration.

The red pigment granules are found in the polyp after the completion of the regeneration, and some of them are somewhat later thrown out. They therefore perish in the process of the formation of the polyp. It is possible that these pigment granules are respiratory pigment; it is, however, also possible that they carry substances other than oxygen to the new polyps. They are consumed in the process of polyp formation and their remains are cast out of the polyps after the regeneration. The fact that they perish in the process of regeneration does not speak against their importance for the formation of polyps, as has been maintained by Morgan, since red blood corpuscles also regularly perish in the liver.

RECAPITULATION OF THE RESULTS.

Former experiments had shown that the polarity observed in the regeneration of Tubularia is not to be referred to a morphological polarity of the individual cells, but to a process which is comparable as to its variety with a process of streaming in the direction of the aboral to the oral pole. This led to the idea that a ligature placed around the middle of the stem must abolish the polarity. This idea was experimentally tested and found to be true. Further experiments on the same subject are being carried on in other organisms.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 18, pp. 163–164. May 23, 1904

THE ACTION OF CASCARA SAGRADA.

(A Preliminary Communication.)

BY

JOHN BRUCE MacCALLUM.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

1. I have shown in previous papers that the saline purgatives act not only when introduced into the stomach or intestine, but also when they are injected subcutaneously or intravenously and when they are applied directly to the peritoneal surfaces of the intestine. It was shown that they act in two ways; by increasing the peristalsis and also by increasing the secretory activity in the intestine. It was found also that both these activities could be suppressed by calcium chloride. The question then arose as to whether the vegetable purgatives act in the same way. The difficulty of obtaining a solution of these purgatives in a fluid which itself was indifferent had first to be overcome.

2. The dried extract of cascara sagrada is only very slightly soluble in distilled water. A mixture in distilled water has a marked acid reaction.

3. If sodium hydrate or sodium bicarbonate be slowly added to this mixture until the acid is neutralised, the cascara is dissolved and a clear dark brown solution is obtained; $\frac{1}{2}$ g. cascara may be readily dissolved in 25 cc. $\frac{m}{25}$ NaH CO_3. This solution may be precipitated by the addition of a few drops of sulphuric acid, and redissolved by again adding NaH CO_3.

4. The cascara extract is thus soluble only in a neutral or alkaline solution, and is precipitated in the presence of acid. It is insoluble in distilled water on account of the free acid which the powder contains.

5. The cascara extract is soluble in the intestinal juice of a rabbit, but is relatively insoluble in gastric juice.

6. The intravenous injection of 1 cc. 2% solution of cascara extract in $\frac{m}{25}$ NaH CO_3 produces within one minute a very active peristalsis. The same dose of $\frac{m}{25}$ NaH CO_3 alone has no such effect.

7. When poured on the peritoneal surfaces of the loops the cascara solution produces strong peristaltic movements after an interval of 2–3 minutes.

8. The injection of 2–3 cc. of the cascara solution into the lumen of the small intestine causes increased peristalsis in 2–3 minutes. When placed in the stomach however no action is to be observed in the first half hour. It is to be assumed that the cascara is precipitated in the stomach and goes into solution only when it reaches the intestine where the acid is neutralised.

9. When administered either subcutaneously, intravenously or applied locally to the peritoneal surfaces of the intestine, cascara solution causes not only increased peristaltic activity but also an increased secretion of fluid into the intestine.

10. The movements caused by cascara may be inhibited only partially by the administration of calcium chloride.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 19, pp. 165–174. June 1, 1904

ARTIFICIAL PARTHENOGENESIS AND REGULAR SEGMENTATION IN AN ANNELID (OPHELIA)[1]

BY

G. BULLOT.

With 13 figures.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

I.

In Echinoderms, Annelids and Molluscs, living larvae have been obtained by artificial parthenogenesis. While, in those Echinoderms and Molluscs which have been investigated, the parthenogenetic larvae came undoubtedly from eggs segmenting regularly, in Annelids they seemed to be derived from unsegmented eggs. The first to call attention to this behavior on the part of the parthenogenetic larvae of Annelids was J. LOEB[2] in his researches on Chaetopterus. He observed that the eggs which produced larvae did not always segment before the cilia made their appearance. Later, F. R. LILLIE,[3] made a cytological investigation of these eggs and found that the nuclear division was abnormal.[4] He noticed, that the ciliated larvae did not have

[1] Reprinted, in part, from the Archiv für Entwickelungs-Mechanik der Organismen. Vol. 18, p. 161, 1904.

[2] J. LOEB; Experiments on Artificial Parthenogenesis in Annelids *(Chaetopterus)* and the Nature of the Process of Fertilization. Am. Journ. Physiol. Vol. IV, p. 423, 1901. J. LOEB, M. H. FISCHER, H. NEILSON, Weitere Versuche über künstliche Parthenogenese. Vorläufige Mittheilung. Pflüger's Archiv, Bd. 87, p. 504.

[3] F. R. LILLIE; Differentiation without Cleavage in the Egg of the Annelid *Chaetopterus pergamentaceus*. Archiv f. Entw.-Mech. Bd. XIV, p. 477, 1902.

[4] Dr. LOEB tells me that he is not convinced that LILLIE's results are typical, and that he considers it possible that, if more attention is given to the treatment of the eggs, more normal phenomena of development may be obtained.

the same shape as the normal ones, and concluded from this that they were not trochophores but mere "ciliated structures" very far behind the real larvae in organization. For two other Annelids, *Amphitrite* and *Nereis* M. H. FISCHER[1] was unable to decide whether or not the parthenogenetic larvae are normal, though his opinion is that, in those forms also, the larvae originate frequently from unsegmented eggs, the segmented eggs generally going to pieces before reaching the blastula stage. The object of the present paper is to show that this is not true for all annelids and that, in one of them at least, the parthenogenetic larvae develop from eggs segmenting regularly.

II.

J. LOEB described two methods which might be used to bring about artificial parthenogenesis. The first one is by increasing the osmotic pressure of the sea-water, the second by adding to the sea-water some substance acting chemically. In my experiments, it was the first method only which was followed. Among the different substances employed to increase the osmotic pressure of the sea-water, KCl was found to be the most effective. NaCl also gave positive results, but the number of swimming larvae obtained was always much smaller than with the former salt. When cane sugar was employed, though it seemed to influence the eggs in the beginning, swimming larvae were never formed. It may be that, for this substance, the right concentration and the right time of exposure have not been determined. Even for KCl the results were not constant at first. This was attributed, in part, to the considerable changes which took place in the temperature during the day. J. LOEB[2] and E. LYON[3] have pointed out the important rôle played by temperature in the experiments on artificial parthenogenesis, principally during the time the solution is acting upon the eggs. In my experiments the temperature varied from 11° C. early in the morning to more than 25° C, in the afternoon, and, as they were started at different

[1] M. H. FISCHER; Further Experiments on Artificial Parthenogenesis in Annelids. Am. Journ. Physiol. Vol. VII, p. 301, 1902. M. H. FISCHER; Artificial Parthenogenesis in Nereis. Am. Journ. Physiol. Vol. IX, 1903.

[2] J. LOEB; Uber Methoden und Fehlerquellen der Versuche über künstliche Parthenogenese. Archiv f. Entw.-Mech. Bd. XIII, 1902, p. 481.

[3] E. LYON; Experiments in Artificial Parthenogenesis. Am. Journ. Physiol. Vol. 9, 1903, p. 308.

hours of the day, the solutions acted upon the eggs at very different temperatures. In order to avoid all the variations which might be due to changes in temperature, it was decided, once for all, to put all the dishes, during the entire course of the experiments, in a bath of running fresh water the temperature of which happened to be remarkably constant. Many days it stood almost exactly at 18° C. from the morning till evening and even during the night. On days in which the weather became cooler the temperature never fell below 17° C.: on warmer days it never rose above 20° C. Under such conditions the best mixture of KCl and sea-water was 20 c.c. 2½ n KCl + 80 c.c. sea-water acting during two hours. More or less than 20 c.c. 2½ n KCl acting during more or less than two hours did not give equally satisfactory results; the number of swimming larvae was less and they seemed less active.

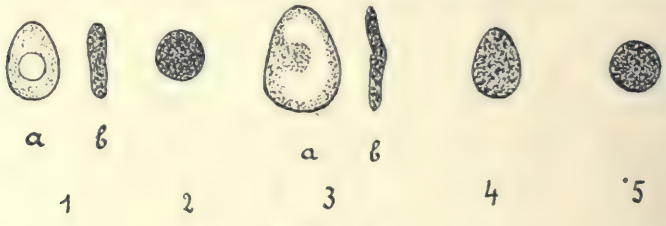
The control eggs which were, in each experiment, always submitted to exactly the same manipulations as the eggs from which larvae were produced, never gave any swimming larvae.

III.

When the eggs of *Ophelia*, as soon as taken from the body, are examined in sea-water, they have the appearance of laterally compressed ovoids of a gray color. Each egg contains a large and very distinct central nucleus (Fig. 1). However this appearance does not persist long. After two hours many of the eggs are modified, but not all in the same way. Some have become black, perfectly spherical, much smaller and are throwing out their polar bodies (Fig. 2). They may occasionally divide into two or four cells, but they do not undergo further development. Instead of giving swimming larvae, they disintegrate, so that, on the following day, each of these eggs is an irregular heap of small spheres. Most of the other eggs which do not turn black become larger, without losing their flat oval shape and their nuclei become invisible (Fig. 3). Some eggs keep their original appearance. On the following day many of the eggs are dark and spherical and disintegrated into heaps of small globules.

These are the changes which may be noted in the unfertilized eggs when kept in normal sea-water from the moment they are

removed from the animal. When such eggs are put into the mixture of 20 c.c. $2\frac{1}{2}$ n KCl + 80 c.c. sea-water for two hours and are then examined in the KCl mixture, they are found to have maintained their normal size and shape, but they are darker and their nuclei have become invisible (Fig. 4). If, then, they



Figs. 1–5.

1. Normal egg immediately after removal from the body. *a* flat surface. *b* profile.

2 and 3. Appearance of the eggs when they have been kept for 2 hours in sea-water. Some are like 2; many like 3.

3*a* flat surface. 3*b* profile.

4. Appearance of the eggs which have remained 2 hours in KCl.

5. Appearance of the same eggs 1 hour after being transferred to sea-water.

are again placed in sea-water and if samples are examined from time to time, the following can be observed. One hour after removal from KCl they are black and spherical (Fig. 5), and are throwing out their polar bodies. After two hours some have already divided into two cells. Then the divisions go on regularly, into four, eight, sixteen and more cells. After five hours the cells are more than sixteen in number, and from this stage on, the individual cells can no longer be recognized. During the following hours the blastula is formed with its characteristic imperfectly spherical shape. After ten hours some blastulae begin to swim. The number of swimming blastulae gradually increases. They always remain at the bottom of the dish. This is in contradistinction to the case of larvae developing from fertilized eggs, which swim through all the layers of the liquid and are much more lively. The parthenogenetic larvae do not live longer than two days. Of the total number of eggs 60% to 80% divide, while only 20% to 60% form swimming larvae. The latter number was obtained but a single time. In general the proportion of swimming larvae is between 25% and 40%. The

eggs which do not divide maintain their spherical form. The eggs which divide and do not develop into swimming trochophores disintegrate after a certain number of divisions.

IV.

Do the swimming blastulae found in the cultures after ten hours arise from the segmented eggs, or do they originate from unsegmented eggs? Since in the case where 80% of the eggs divided, 60% of the total number of eggs gave rise to swimming larvae, it is clear that at least 40% of the total number of eggs must have given rise to larvae through segmentation. This argument does not hold good for the cases in which 25% of larvae were obtained and only 60% of the eggs segmented, because all larvae could have originated from the 40% of undivided eggs. And even if all the cases were like the first one, it would still be desirable to make sure by direct observations that the larvae developed from segmented eggs. In order to determine this point, a certain number of eggs were placed in a flat-bottomed watch-glass and covered with a thin layer of sea-water. Their progressive transformations were then watched under the microscope. To avoid the displacement of the eggs through vibration and mechanical shocks, the microscope was placed upon the cement pier of the balance room. It is easy to see from the following drawings, made by aid of the camera lucida, that the eggs kept their respective positions in such a manner that there was no difficulty in identifying each one of them.

The camera drawings I-VIII represent a number of eggs after treatment with 2½ n KCl, as seen at different times. In all drawings the same figure refers to the same egg.

These drawings show:

1. That among the 48 eggs which were in the field 16 gave larvae all originating from segmented eggs. These eggs are: 1, 3, 5, 7, 8, 9, 10, 12, 16, 19, 23, 28, 32, 42, 47.

2. That 32 eggs divided, that half of them produced the 16 larvae, and that in the other half development was as a rule arrested when they had attained the 2-, 3-, or 4-cell stage. These eggs are: 4, 13, 14, 21, 26, 27, 31, 33, 34, 37, 40, 43, 44, 45, 46, 48.

3. That 16 eggs did not divide, but maintained their spherical shape or partially desintegrated. These eggs did not give rise to larvae.

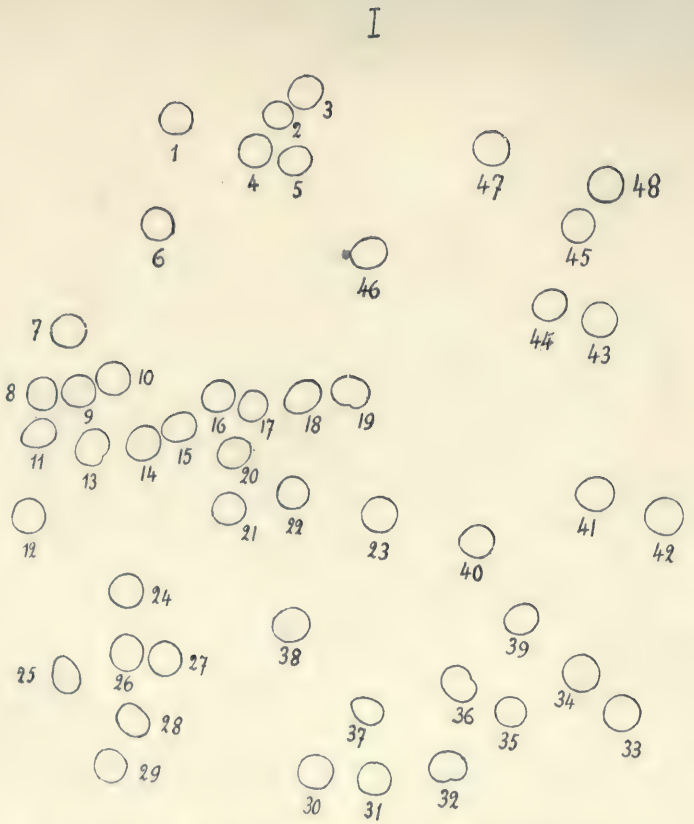
This series of observations was repeated on four other cultures with similar results.

Other observations, made on the development of larvae from fertilized eggs, showed that it was identical with that of the parthenogenetic larvae. The fertilized eggs divided in quite the same way. The only differences were in the rapidity of the development, in the proportion of eggs giving swimming larvae, and in the longevity of these larvae. When the eggs are fertilized, they divide more quickly and about 95% give swimming larvae. These larvae can be kept alive during several weeks, if the sea-water does not undergo putrefaction. They are also more lively than the parthenogenetic larvae.

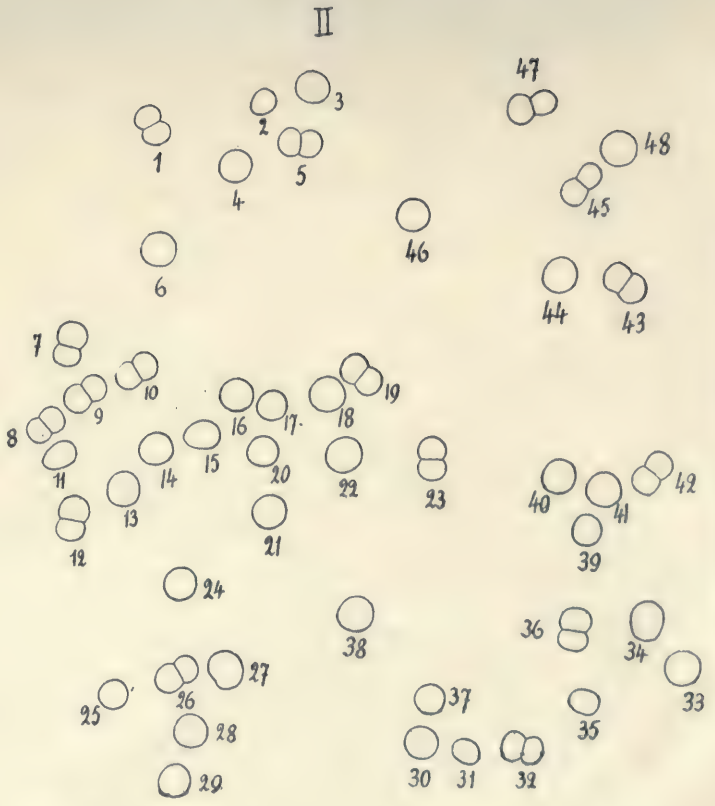
These experiments show conclusively that in the Annelid of the genus *Ophelia*[1] which was used in these experiments the parthenogenetic larvae originate from regularly segmenting eggs.

I wish to express my thanks to Prof. O. P. Jenkins of the Leland Stanford Junior University for the opportunity accorded me of working at the Hopkins Seaside Laboratory, where this investigation was carried on.

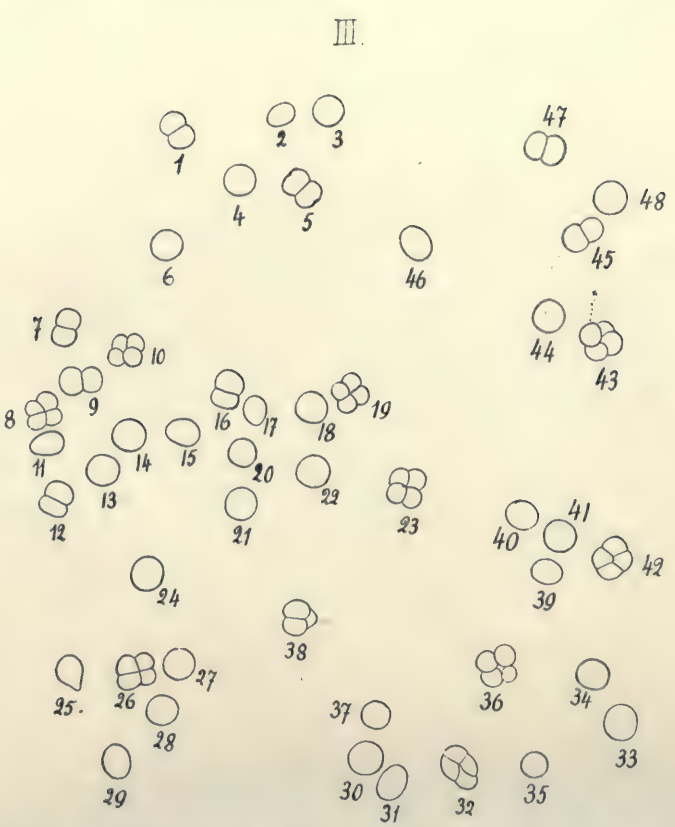
[1] Dr. H. P. Johnson was kind enough to examine specimens of the marine worm whose eggs were used in the present paper. He classified it in the genus *Ophelia* and thought that it was probably a new species.



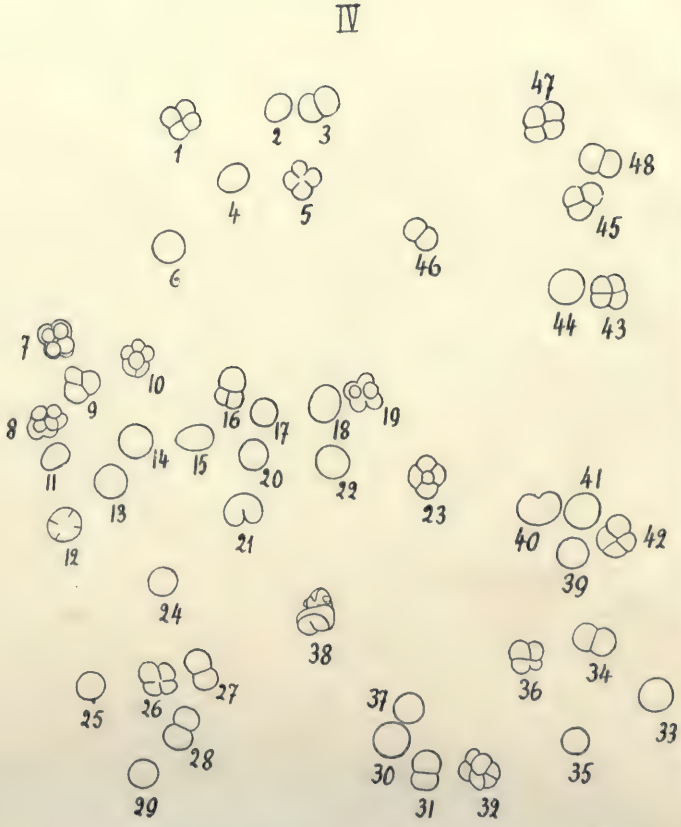
I. Appearance of the eggs one hour after being transferred to sea-water. Many have become spherical. Some show the beginning of a segmentation into 2 cells (13, 19, 32, 36).



II. The same egg an hour later. A certain number are in the 2 cell stage. The other ones are still undivided.



III. The same eggs half an hour later. A certain number are in the 4 cell stage.

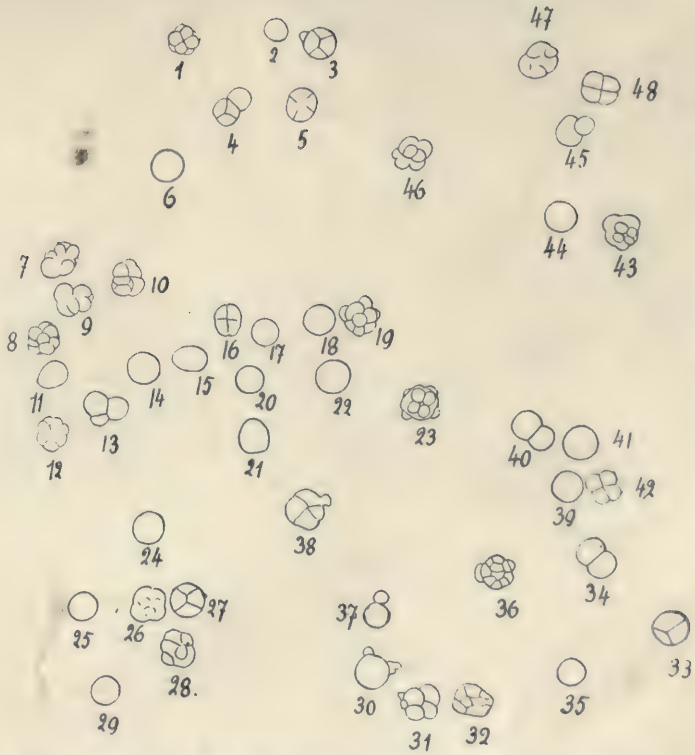


IV. The same eggs half an hour later. Two are in the 8 cell stage. A certain number in the 4 cell stage.

V

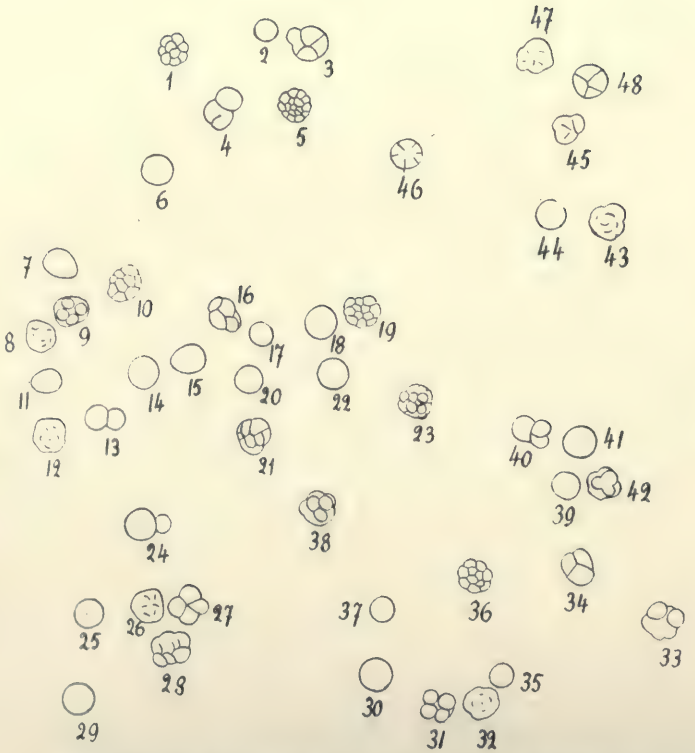
V. The same eggs three quarters of an hour later. A certain number are in the 8 or 4 cell stage.

VI.



VI. The same eggs half an hour later. A certain number are divided into more than 8 cells.

VII



VII. The same eggs one hour later. A certain number are in the blastula stage. A certain number in the morula stage.

VIII

VIII. The same eggs two hours later. A certain number are in the blastula stage.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 20, pp. 175-185. July 10, 1904

ON THE ACTION OF SALINE PURGATIVES IN RABBITS AND THE COUNTERACTION OF THEIR EFFECT BY CALCIUM.[1]

(Second Communication.)

BY

JOHN BRUCE MacCALLUM.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

I.—The Mechanism of the Action of Saline Purgatives.

In attempting to explain the action of saline purgatives, Schmiedeberg[2] states that these salts are absorbed with difficulty, and hence reach the lower part of the intestine unchanged. In the large intestine, according to his theory, the salts prevent the faeces from becoming compact by inhibiting the absorption of fluids from the lumen. This hypothesis is supported by Wallace and Cushny,[3] who add that the absorption of fluids from the intestine is retarded especially by the salts of those acids which tend to form insoluble salts with calcium. Hofmeister[4] found that gelatin plates absorb less water when soaked in solutions of sodium sulphate, tartrate, citrate, etc., than they do when soaked in chlorides or bromides. Schmiedeberg's theory of the action of

[1] Reprinted from the American Journal of Physiology, Vol. X. November 2, 1903, No. 3, p. 101. A preliminary report of the results of these experiments was published in the University of California Publications, Physiology, May 25, 1903, Vol. 1, No. 2, p. 5.

[2] SCHMIEDEBERG: Arzneimittellehre, Leipzig, 1883.

[3] WALLACE and CUSHNY: American Journal of Physiology, 1898, I, p. 411.

[4] HOFMEISTER: Archiv für experimentelle Pathologie und Pharmakologie, 1888, XXV, p. 1.

purgatives has been widely accepted. The older idea of Liebig that the salt solution attracts fluid from the blood into the lumen of the intestine on account of its osmotic pressure has long been abandoned. The purgative action of the salt solution does not increase with an increase in its concentration (Buchheim). Loeb,[1] while not denying the possibility of the inhibiting action of the saline purgatives on absorption, states that these salts are identical with those which produce contact irritability, muscular twitchings, and hypersensitiveness of the nervous system. Further, he suggests that the increased peristalsis may be due to an increase in the irritability of the nerves and muscles of the intestines.

In order to decide this question, and to render more clear the action of saline purgatives, I have made a series of experiments on rabbits, testing a number of salts, including sodium citrate, sulphate, tartrate, oxalate, phosphate, and fluoride, barium chloride, and magnesium sulphate. It was found that the effects of the drugs could be most satisfactorily studied by exposing the intestines and observing them directly in animals under the influence of morphine. Subcutaneous injection of 5 c.c., 1 per cent solution of morphine proved sufficient for surgical anaesthesia, without affecting the intestinal movements in the rabbits which were used. These weighed on an average twelve hundred grams. In addition to this method, many experiments were made in which animals were kept in separate cages, and the amount and character of the faeces observed during several hours after the administration of the salts.

It was found that *all those salts which act as purgatives when introduced into the stomach or intestine, have the same action when injected subcutaneously or intravenously.* The injection of 1 to 2 c.c. $\frac{m}{8}$ sodium citrate solution[2] into the jugular vein of a rabbit causes a striking increase in the peristaltic movements. This activity begins in from 1 to 2 minutes after the injection. Loops of the intestine which before the injection lay quiet

[1] Loeb: Decennial Publications of the University of Chicago, 1902, p. 10.

[2] The salt solutions were made up with distilled water as fractions of molecular solutions, Thus $\frac{m}{8}$ sodium citrate solution means $\frac{1}{8}$ molecular weight of the salt in grams (including water of crystallization) dissolved in 1000 c.c. water.

and collapsed, are set in active motion. They become rounded and prominent, and seem to occupy a greater volume. The fact that the animal is under the influence of morphine does not interfere to any extent with this action. Claude Bernard[1] has stated that sodium sulphate introduced into the veins acts as a purgative; and that the same action is obtained by subcutaneous injection of magnesium sulphate.

When introduced into the intestine or stomach a much larger quantity of the salt is required to produce an equal effect, and the action takes place only after an interval of from 10 to 15 minutes. By piercing the wall of the intestine with a hypodermic needle, and forcing 5 to 10 c.c. $\frac{m}{8}$ sodium citrate solution into the lumen, a similar increase in peristalsis is brought about. The movements begin in about 10 to 15 minutes after the injection, not only in the loops of intestine which contain the solution, or the loops near the stomach, but simultaneously in all parts of the intestine.

When given subcutaneously, these salts do not act immediately, but only after an interval of 10 to 15 minutes. By this method usually a fairly large quantity, *e.g.*, 10 c.c. $\frac{m}{8}$ sodium citrate solution is necessary to produce increased peristaltic movements. When the actual passage of faeces is observed, relatively large doses also are necessary. No appreciable effect is obtained with less than 10 c.c. $\frac{m}{8}$ sodium citrate solution given subcutaneously. A number of control animals were kept in each case, and the average weight and character of the normal faeces carefully determined. During the first six hours following the injection of the salt, the faeces were collected and weighed. The purgative effect usually takes place during the first hour. Considerable variation in the action is to be observed in different rabbits; but with subcutaneous injection of the salt, there is constantly a marked increase in the weight of the faeces varying from two to six times the average normal weight. The faeces are sometimes of a semi-fluid character, a fact which is the more striking because the normal faeces of rabbits are drier and more definitely formed than the dejecta of most other animals.

[1] Claude Bernard: Leçons sur les effets des substances toxiques et médicamenteuses, Paris, 1857.

Schmiedeberg's theory was an effort to explain this increase in the fluid contained in the faeces following the administration of a saline purgative. That the prevention of the absorption of fluids from the intestine is not the main cause of the production of faeces containing much fluid is shown by the following facts: In addition to the increase in peristalsis caused by the administration of the saline purgatives (whether subcutaneous, intravenous, or intraintestinal) *there is to be observed also a more or less marked increase in the secretion of fluid into the intestine.* A short time (20 to 30 minutes) after the salt is given there is usually found in the intestine a considerable quantity of a clear yellow fluid. This does not resemble bile. The collapsed loops of intestine, in addition to being set in active squirming motion, become gradually filled with fluid after the purgative is administered.[1] The actual passage of faeces takes place usually within an hour after the intravenous injection of the purgative. The exact nature of the fluid secreted into the intestine has not been studied, and the details of its secretion remains to be investigated. It was noticed also in a number of cases that an increased flow of saliva and urine occurred when one of these salts was introduced into the body. These problems of secretion are still to be studied. After the subcutaneous administration of sodium fluoride solution, the salivation in two or three cases was so marked that the saliva fell in drops to the floor. In overdoses of sodium citrate, the same phenomenon was observed in several cases; while it is well known that barium chloride causes salivation. Repeated urination is a fairly constant accompaniment of the subcutaneous and intravenous injections of all of these salts.

In connection with the production by these purgative salts of an increased flow of fluid into the intestine, together with salivation and increased urination, it is interesting to note the subcutaneous and intravenous administration of pilocarpine and physostigmin as purgative by veterinarians. These drugs act primarily upon the glands of the body, and no doubt owe their purgative action in part to the production by them of increased secretion of fluids into the intestine.

[1] As quoted by Schmiedeberg *(Loc. cit.)*, Bunge has stated that sodium phosphate in rabbits causes an increase in the fluids contained in the alimentary canal. I have been unable to find Bunge's original statement.

The action of the various salts which act as purgatives when administered subcutaneously or intravenously varies in intensity; but it is difficult to make a definite list in which they can be placed in order of increasing strength. Barium chloride is the most powerful of all. The action is rapid, and with very small doses marked purgative effects are obtained. It is well known among veterinarians as a powerful purgative, and is commonly administered by them intravenously. An intravenous injection of 0.75–1 gram is sufficient to purge a horse weighing one thousand pounds. The intensity of action of sodium citrate, fluoride, sulphate, tartrate, oxalate, and phosphate decreases approximately in the order named. Magnesium sulphate, when given subcutaneously, is no less active than sodium sulphate, but has a somewhat more poisonous effect than sodium sulphate or sodium citrate. Large subcutaneous doses often cause death in a very short time in rabbits, while equal doses of sodium sulphate seem to be harmless. As a subcutaneous purgative, it is not to be recommended. Barium chloride also produces unfavorable symptoms. Rabbits often do not recover from a relatively small dose. Sodium salts, however, such as the sulphate, citrate, tartrate, etc., may apparently be given subcutaneously with impunity. With regard to the common use of barium chloride by veterinarians, attention must be called to its dangerous nature, and to the possibility of the occurrence of serious after-results.

In addition to the purgative effects of these salts, there is produced by the injection of sodium citrate, tartrate, fluoride, oxalate, and phosphate a condition of extreme hypersensitiveness of the muscular and the nervous system, which is entirely analogous to that described by Loeb[1] in isolated muscles, motor nerves, and the skin of frogs. The muscular twitchings and increase in nervous excitability which Loeb caused in isolated muscles and in living frogs by means of these salts, I have been able to produce to a marked degree in rabbits by the subcutaneous injection of the same solutions. Subcutaneous injection of 10 c.c. $\frac{m}{1}$ sodium citrate solution produces within half an hour well-marked twitchings of the muscles in all parts of the body,

[1] Loeb: *Loc. cit.*

more noticeable in those of the gluteal region. These are accompanied by tetanic contractions of the limbs, and in some cases by general convulsions of varying intensity. There is always a hypersensitiveness of the skin, and all the reflexes are much exaggerated. One of the first effects to be noticed is an incoordination of the movements of the hind limbs. If the animal is held up by the ears, the feet tremble, and, if touched, the hind limbs jerk away violently, or become rigid. The muscular twitchings appear immediately at the place of injection, but only after 20 to 25 minutes on the opposite side of the body.

In animals to which repeated small doses of sodium citrate are given, a chronic state of hypersensitiveness may be produced. Daily doses of 5 c.c. $\frac{m}{1}$ sodium citrate solution were given to a rabbit throughout one month, and then discontinued. A condition of marked hypersensitiveness persisted for four weeks after the last dose was given. In such an animal muscular twitchings may be produced on the side of the body opposite to the injection in as short a time as two minutes, while in a normal animal it takes 20 to 25 minutes.

To recapitulate, then, those salts which produce muscular twitchings and hypersensitiveness of the nervous system, produce also increased peristalsis, and increased secretion of fluid into the intestine. They produce this purgative effect not only when introduced into the intestine, but also when injected subcutaneously or intravenously. *The presence of the salt in the intestine is therefore not necessary for its cathartic action.* When introduced into the intestine, it takes much longer to act, and requires a much larger dose than is needed when injected into the blood. This seems to indicate that *the salt must first be absorbed into the blood before it can act on the intestine.* The essential feature in the action of saline purgatives is not their presence in the lumen of the intestine, but their absorption into the blood, and the production by them of a condition of hypersensitiveness of the nervous system controlling the intestine. It is difficult to say which part of the nervous system is specially affected. It is possible that the muscle and gland cells themselves are influenced; and it seems justifiable to suggest that the action of these salts is not a specific one, but that they cause a

more general hypersensitiveness. It is difficult to disprove Schmiedeberg's theory, that the absorption of fluids from the intestine is retarded by these salts; but it is certain that the main factor in the production of fluid or less solid faeces is the increased secretion of fluid into the intestine. Since an increased secretion is caused by the absorption of the salt, the method commonly employed in determining the rate of absorption of fluids from the intestine must be defective because they consist in measuring the fluid contained in a certain loop before and after the introduction of a measured quantity of salt solution. This quantity would remain relatively large, not because of a retardation of the absorption, but on account of the increased flow of fluid into the intestine caused by the absorption of the salt.

II.—The Effect of Calcium in Opposing the Action of Saline Purgatives.

It has already been shown by Loeb that the muscular twitching and hypersensitiveness of the nervous system produced by sodium citrate, etc., may be inhibited by calium salts. I have found that a complete analogy exists between this action and the production and inhibition of peristaltic movements in the intestine. *Active peristalsis produced by the intravenous injection of a minimal dose of any purgative sodium salt can be almost entirely suppressed by the subsequent injection of an equal quantity of* $\frac{m}{8}$ *calcium chloride solution.* This counteracting effect takes place within 1 to 2 minutes after an intravenous injection, and in 10 to 20 minutes after a subcutaneous injection, or the introduction of the solution into the intestine or stomach. A much larger quantity is required when introduced subcutaneously or into the alimentary canal. To illustrate this effect, one of a large number of experiments may be cited. To two similar rabbits, equal doses of morphine were administered. The abdominal cavities were opened, and in each case $1\frac{1}{2}$ c.c. $\frac{m}{8}$ sodium citrate solution were injected into the jugular vein. Active peristalsis set in in both animals. After five minutes $1\frac{1}{2}$ c.c. $\frac{m}{8}$ calcium chloride solution were injected into the jugular vein of one animal, while the other was left without further

treatment. Almost immediately the peristaltic movements of the intestine of the animal which received the calcium chloride ceased. Those of the other animal continued. Usually after the injection of calcium the intestines remain motionless for one-half to 1 hour, while in some cases slight peristaltic movements begin after 5 to 10 minutes. This depends largely upon the relative amounts of citrate and calcium chloride solutions which have been administered. It is possible after the movements have been entirely inhibited by calcium chloride to make them active again by a second injection of one of the purgative sodium salts. It is unimportant whether the intestines are set in motion by sodium citrate, sulphate, tartrate, phosphate, or oxalate. The calcium chloride has the same inhibiting effect in all cases. The peristaltic movements produced by barium chloride are usually not stopped by the administration of calcium.

As suggested by Dr. Loeb, the salts which produce rhythmical contractions in voluntary muscle are those which are liable to decrease the concentration of the free calcium ions. The same is true of the salts which act as purgatives; and the hypothesis that their action is due to the removal of calcium from the tissues, or from the fluids bathing them, is a plausible one. It is certain that the subsequent addition of calcium to the tissues restores them to the condition in which they existed before the injection of the purgative salt, and entirely counteracts the purgative effect. It is only on these grounds, and from the fact that these salts in some cases precipitate calcium, that it is possible to state that the purgative action is caused by the removal of calcium from the tissues.

It is not possible to make the general statement that the anion stimulates and the kation inhibits the muscular contractions. In a number of salts it is clearly the anion which causes the purgative action and the muscular contractions, *e.g.*, sodium citrate, sulphate, tartrate, fluoride, etc.; while in other cases it is equally clear that the kation has the same stimulating action, *e.g.*, barium chloride. The kation of calcium chloride on the other hand has an inhibiting effect. That this action also is not dependent upon the valency of the ions is shown by the fact that barium chloride, sodium fluoride, and sodium citrate all have the

same effect, while barium chloride and calcium chloride have effects which are directly opposed to one another. Moreover, the effects produced by magnesium and sodium sulphate on the intestine are similar.

Little can be said at present concerning the practical application of these facts in the treatment of disturbances of the human intestine. *The administration of calcium, however, seems rational in cases of persistent diarrhoea, especially in those cases accompanying hysteria or any disturbance of the nervous system.* And since calcium itself has an irritating action when given in large doses, the treatment would seem to be contraindicated in cases in which the diarrhoea is caused by an inflammation of the mucous membrane of the intestine. The empirical use of calcium, usually in the form of chalk, is commonly resorted to in cases of diarrhoea, its action being explained by the statement that it reduces the acidity of the intestinal contents. But it is evident that it can only act through the calcium, since calcium chloride is more efficacious even than chalk.

In addition to this, the fact that a chronic hypersensitive condition of the muscular and nervous tissues may be brought about in rabbits by the continued administration of salts which tend to diminish the concentration of calcium ions, suggests that the addition of calcium to the tissues might be of service in the treatment of similar conditions in human beings. Those conditions which most nearly resemble that produced in rabbits are hysteria, neurasthenia, and the allied states. Whether there is in reality any analogy, and whether calcium will prove of benefit in such cases, must be the subject of clinical investigation.

The question arises also as to the possibility of administering purgatives to human beings subcutaneously or intravenously. Although in general such methods would be contraindicated, certain cases might arise in which it would be of distinct advantage. The present experiments seem to indicate that subcutaneous or intravenous administration of some of the salts, especially sodium citrate or sulphate, might be safely resorted to. Neither barium chloride nor magnesium sulphate should be given in this way.

III.—Conclusions.

1. In general, the saline purgatives act not only when introduced into the intestine, but also when injected subcutaneously or intravenously.

2. The intensity of their action is greatest with barium chloride and decreases approximately in the following order: barium chloride, sodium citrate, fluoride, sulphate, tartrate, oxalate, and phosphate.

3. The purgative action of these salts is caused, first, by an increase in peristalsis, and, second, by increased secretion of fluid into the intestine, both of which can be directly observed.

4. Although I have not specially studied the secretion of other glands, I have noticed that in a number of cases an increased flow of saliva and urine occurred when these salts were introduced into the body.

5. Intravenous injection of 1 to 2 c.c. $\frac{m}{8}$ solution of these salts causes increased peristalsis within 1 minute. When introduced into the intestine, it takes 10 to 15 minutes, and five times the amount to produce an equal effect.

6. This seems to indicate that even when these salts are introduced into the intestine, they must be absorbed into the blood before they can produce their purgative effect, and that they affect the intestine by increasing the irritability of the nerves and muscles, as Loeb has suggested. Their action in producing less solid faeces is not due to the prevention of the absorption of fluids from the intestine, but to the production of an increased secretion of fluid into the intestine.

7. By the continued administration of small doses of sodium citrate, a chronic condition of hypersensitiveness of the nervous system may be brought about in rabbits, which persists for a considerable time after the drug is discontinued.

8. By the injection of solutions of calcium chloride, the peristalsis caused by these salts can be entirely inhibited.

9. There is a perfect analogy between these actions and the production and suppression of muscular twitchings and nervous hypersensitiveness.

10. The administration of calcium is, therefore, rational, especially in those cases of diarrhoea in human beings which accompany hysteria or nervous excitability of any sort.

The study of this subject was suggested to me by Professor Loeb, and it is a pleasure to thank him for the interest which he has taken in the experiments, and for many helpful suggestions.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 21, pp. 187-197. July 10, 1904

ON THE LOCAL APPLICATION OF SOLUTIONS OF SALINE PURGATIVES TO THE PERITONEAL SURFACES OF THE INTESTINE.[1]

BY

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In a previous paper[2] I have described the effects of subcutaneous and intravenous injections of purgative salts on the intestines of rabbits, and have shown how the peristalsis produced thereby can be inhibited by similar injections of calcium chloride solution. It was further pointed out that these actions are analogous to the production and suppression of rhythmical contractions in voluntary muscles as described by Loeb.[3] This analogy is still more clearly shown by a series of experiments which I have since made on the local application of these salts to the intestine.

I have found that all those salts which produced increased peristalsis when administered intravenously or subcutaneously, or when introduced into the intestine or stomach, have the same action when applied locally to the peritoneal surface of the intestine. The immediate effect of this is essentially local: only those loops which are moistened by the saline solution are at once set in motion. After a short time, the other loops also become

[1] Reprinted from the American Journal of Physiology, Vol. X, January 1, 1904, No. 5, p. 259.

[2] MacCallum, J. B.: American Journal of Physiology, 1903, x, p. 101; also Preliminary Report, University of California Publications, Physiology, 1903, i, p. 5.

[3] Loeb, J.: Archiv für die gesammte Physiologie, 1902, xci, p. 248.

active. Peristalsis thus produced may be entirely inhibited by the local application of a solution of calcium or magnesium chloride. This may be illustrated by the following experiment:—

The intestines of a rabbit under the influence of morphine were exposed. On a small group of coils there were poured 3 c.c. $\frac{m}{8}$ sodium citrate solution. Almost immediately (within 1 minute) the loops became very active. Strong contractions of the muscle coats took place. After a few minutes, the other loops were also set in movement, so that the whole small intestine showed active peristalsis. The citrate solution was then washed off by $\frac{m}{8}$ NaCl solution, and about 3 c.c. $\frac{m}{8}$ $CaCl_2$ solution poured on the loops. The peristaltic movements were promptly suppressed, and the intestine remained quiet. By the further addition of citrate solution, the coils were set in active movement once more, and by the subsequent application of calcium chloride solution again inhibited. This was repeated many times (sixteen) and apparently might have been continued as long as the intestine remained alive.

The same results were obtained by using instead of the sodium citrate solution, a solution of barium chloride, sodium sulphate, fluoride, bromide, iodide, phosphate (Na_3PO_4), oxalate or tartrate. Local application of solutions of any of these salts produces increased peristaltic activity. Solutions of sodium chloride have a very slight action of the same character. On the other hand, the intestinal movements are equally inhibited by calcium chloride and magnesium chloride, while strontium chloride has a similar but less powerful inhibiting action.

In testing those salts with which it was necessary to use dilutions greater than $\frac{m}{8}$, the dilution was made with a neutral fluid consisting of sodium chloride and magnesium chloride. It was found that $\frac{m}{8}$ NaCl solution increased to a slight extent the peristaltic movements. By adding to 10 c.c. $\frac{m}{8}$ NaCl, 0.5 c.c. $\frac{m}{8}$ $MgCl_2$, a fluid was obtained which had apparently neither stimulating nor inhibiting effects. In addition to solutions made up by dilution with this neutral fluid, others were used in which the salt solutions were diluted with distilled water. Practically the same results were obtained in both cases. It was found that 1 c.c. $\frac{m}{320}$ $BaCl_2$ solution applied locally to the intestine is suffi-

cient to cause strong peristaltic movements in a rabbit. This quantity contains about 0.00076 gm. barium chloride. In the case of sodium citrate, the concentration must be considerably greater. No solution of this salt more dilute than $\frac{m}{80}$ is active in a rabbit. Of all the purgative salts, barium chloride is by far the most powerful. If a drop of $\frac{m}{8}$ $BaCl_2$ be placed on the serous surface of an intestinal loop, or if a small area be moistened with this solution by means of a camel's hair brush, the muscle beneath the moistened area will almost immediately contract so that a ring-like constriction of the intestine is formed. This often is so sharply marked that it suggests the effect produced by tying a ligature around the intestine. This constriction remains for a few moments, and then gradually moves along the loop in the direction of the normal peristalsis. If the solution be injected into the muscle of the intestine at any point with a hypodermic needle, a similar sharp constriction takes place. If also a few drops be injected directly into a branch of the superior mesenteric artery, all that part of the loop supplied by the arterial branch will contract violently. These statements are true also in the case of sodium citrate, fluoride, sulphate, etc.; the action of these salts, however, is less powerful.

It must be added here also that the actual passage of faeces may be produced within an hour by the application of the purgative salts to the serous surfaces of the intestine. This takes place most quickly with barium chloride. It is possible to observe directly through the semi-transparent walls of the intestine the rapid passage of faecal masses from one loop to another.

The intestines of the rabbit are apparently much more sensitive to the action of sodium citrate and sulphate than are those of the dog or cat. Barium chloride, on the contrary, acts with equal strength in all these animals. In a rabbit, the intestines are always set in active peristaltic movement by contact with $\frac{m}{8}$ sodium citrate solution; and even much more dilute solutions are, as a rule, effective. In a cat, however, it was found that a $\frac{m}{8}$ solution of sodium citrate has practically no effect, while a $\frac{5m}{8}$ solution sets the intestine in active motion. In a dog also $\frac{m}{8}$ sodium citrate solution is usually ineffective. Similarly a $\frac{m}{8}$ sodium sulphate solution is inactive in a dog while a $\frac{m}{2}$ solu-

tion of the same salt starts up distinct peristalsis. In the cat and dog, also, the peristalsis may be inhibited by calcium or magnesium chloride, as shown in the following experiments: The intestines of a cat were exposed in the usual manner, and a $\frac{m}{8}$ solution of sodium citrate was applied to the serous surface of the loops. There was no increased movement. There were then poured on the loops a few cubic centimetres of a mixture of 5 c.c. $\frac{5m}{8}$ sodium citrate and 5 c.c. $\frac{5m}{8}$ $CaCl_2$. The loops remained motionless. After waiting a considerable time (15 minutes), a $\frac{5m}{8}$ solution of sodium citrate alone was poured on the intestine. Almost immediately they became very active; and the peristalsis continued until calcium chloride was again applied. The loops then came to a standstill. The difference in susceptibility to the action of citrate which exists between rabbits on the one hand, and dogs and cats on the other, may be in some way connected with their being herbivorous and carnivorous animals respectively.

The action of the sodium citrate, sulphate, fluoride, etc., when applied locally, may be inhibited by the administration of an approximately equal quantity of calcium or magnesium chloride of the same concentration. The counteraction of the effect of barium chloride, however, requires a much greater concentration of calcium. Using equal quantities of the two salts, the action of the barium is usually not inhibited, a fact which I have previously stated. With greater concentrations of the calcium chloride, the antagonistic action, however, is clear. This is shown in the following experiment: Applied locally to the intestine of a rabbit 1 c.c. $\frac{m}{320}$ $BaCl_2$ solution caused active peristaltic movements. The application of 1 c.c. $\frac{m}{320}$ $CaCl_2$ solution exercised no inhibiting effect whatever. The same quantity of $\frac{m}{40}$ $CaCl_2$ was then poured on the loops, and a slight but distinct quieting of the loops took place. The addition of 1 c.c. $\frac{m}{8}$ $CaCl_2$ caused the loops to become entirely motionless. After waiting a considerable time, 1 c.c. $\frac{m}{8}$ $BaCl_2$ was poured on the intestine, Immediately violent peristaltic movements took place. Several c.c. $\frac{m}{8}$ $CaCl_2$ exercised practically no inhibiting influence; while 2 c.c. $\frac{5m}{8}$ $CaCl_2$ solution suppressed the movements entirely for a short time.

The question concerning the exact seat of action of the purgative salts remains still unanswered. Whether, upon being absorbed into the blood, they act on the central nervous system is not known. There is no evidence to show that this is the case. It seems certain, on the other hand, from the experiments here described, that they undoubtedly have a peripheral action either on the peripheral nervous mechanism or on the muscle cells themselves. It is impossible to prove that there is no action on the central nervous system, and at present it seems impossible to prove whether the peripheral action is directly on the nerves or on the muscles. The existence in the walls of the intestine of the ganglionic plexuses of Auerbach and Meissner must be taken into consideration; and with the methods available there seems to be no way of distinguishing the action on these plexuses and the direct action on the muscle cells. The ultimate effect is on the muscles and glands; and the fact that an entirely local ring-shaped constriction can be brought about by the local application of a drop of one of the salt solutions to the surface of the intestion would seem to indicate that only a small group of the circular muscle fibres themselves is affected. The fact that the nerve plexuses form a continuous net work, and are intimately related in their various parts, would suggest that the occurrence of an action on these plexuses confined to so small an area is improbable. The discussion of the exact location of the action is, however, of relatively little importance, as compared with the main facts shown by these experiments, namely, that *it is possible to produce, by the local application of a purgative salt to the serous surface of the intestine, a striking increase in peristalsis, and to suppress these movements by a similar application of solutions of calcium, magnesium, or strontium chloride.*

II.—The Production of Increased Secretion of Fluid into the Intestine by the Saline Purgatives.

I have already stated[1] that, following the subcutaneous or intravenous administration of a saline purgative, a distinct increase in the secretion of fluid into the intestine takes place. Although this increase can be directly observed, a series of

[1] MacCallum, J. B.: American Journal of Physiology, 1903, x, p. 101.

experiments had been planned by which the relative quantities of fluid secreted into an isolated loop of the intestine, before and after the administration of a saline purgative, might be determined by actual measurements. The experiments were interrupted by the summer vacation, and could not be included in the previous paper. Since then, however, I have been able to make these determinations with the following results.

For these experiments rabbits and dogs were used. A loop of considerable length was tied off, and a cannula inserted in its lower end. In the rabbit, the upper part of the small intestine was used, since in this region of the alimentary canal the secretion of fluid seems to be most active. The upper ligature was placed in each case below the entrance of the common bile duct, while the lower ligature and the cannula were placed from twenty-five to thirty centimetres below. By gently lifting the successive loops, the fluid could be easily drained through the cannula. This is made more easy by placing the animal board at a considerable angle with the table. After the loop was emptied, the cannula was closed by a clamp, and the normal secretion allowed to collect in the loop for a period of 10 minutes. The contents were then removed and measured. This was repeated for successive periods of 10 minutes, and an estimate of the normal rate of secretion was thus obtained in each case. The purgative salt was then administered either subcutaneously or locally. In the latter case, the solution was allowed to drop from a pipette upon the loop. Care was taken to have the saline solutions at the body temperature, and approximately isosmotic with the blood of the animal. Ten minutes after the administration of the purgative, the loop was again emptied and the contents measured. This was repeated several times. In each case special care was taken to have no interval between the emptying of the loop and the beginning of the succeeding period of 10 minutes. In other words, the loop was always entirely empty at the beginning of each period. The operations were performed under morphine; the rabbits were given 5 c.c. 1 per cent solution of the hydrochlorate subcutaneously, and the dogs received the same dose of morphine, and, if necessary, also ether.

The results of the experiments may be seen from the following reports:

1. Rabbit. Loop 30 cm. long, upper part of small intestine.

Loop contained in beginning[1]	5.0 c.c.
Fluid removed after 1st 10 minutes	0.2 c.c.
" " " 2d " "	0.5 "

2 c.c. $\frac{m}{8}$ $BaCl_2$ injected subcutaneously.

Fluid removed after 1st 10 minutes following injection	4.0 "
" " " 2d " " " "	3.4 "
" " " 3d " " " "	3.0 "

In this rabbit the increased secretion of fluid was accompanied by extremely active peristaltic movements. The faeces could be seen passing along the loops of the lower part of the intestine with great rapidity. Within 30 minutes after the administration of the salt, the passage of faeces to the outside began. This continued for some time, the faeces becoming constantly softer, until finally they were almost entirely unformed.

As shown by this experiment, and also by the following ones, the action of barium chloride persists for a considerable length of time. The action of sodium citrate is more transitory.

2. Rabbit. Loop 25 cm. long.

Loop contained in beginning	3.0 c.c. fluid deeply bile stained.
After 1st 10 minutes	1.0 " " " " "
" 2d " "	0.8 " " somewhat lighter in color.

Injected 2 c.c. $\frac{m}{8}$ $BaCl_2$ solution subcutaneously.

After 1st 10 minutes following injection	2.5 c.c. fluid light yellow.
After 2d 10 minutes following injection	1.6 " " very light yellow.
After 3d 10 minutes following injection	1.8 " " almost colorless.
After 4th 10 minutes following injection	1.6 " " quite colorless.
After 5th 10 minutes following injection	1.0 " " " "

3. Rabbit. Loop 32 cm. long.

Loop contained in beginning	6.0 c.c.
After 1st 10 minutes	0.4 "
" 2d " "	0.1 "
" 3d " "	0.4 "

Poured 5 c.c. $\frac{m}{8}$ sodium citrate on loop.

After 1st 10 minutes	6.2 "
" 2d " "	2.0 "

[1] In all these experiments there was no interval between the emptying of the loop and the beginning of the ten minute period which followed. The injections were made as rapidly as possible, and in no case occupied more than a minute.

4. Rabbit. Loop 30 cm. long.

Loop contained in beginning	5.0 c.c.
After 1st 10 minutes	1.6 "
" 2d " "	1.0 "

Poured 3 c.c. $\frac{m}{8}$ sodium citrate on loop.

After 1st 10 minutes	1.6 c.c.
" 2d " "	0.2 "
" 3d " "	0.4 "
" 4th " "	0.4 "

Poured 3 c.c. $\frac{m}{8}$ sodium citrate on loops.

After 1st 10 minutes	1.4 "

5. Dog. Loop 35 cm. long.

Loop contained no fluid, *i.e.*, none could be drained off.

After 1st 10 minutes	0.0 c.c.
" 2d " "	0.0 "
" 3d " "	0.0 "

Poured 3 c.c. $\frac{m}{8}$ $BaCl_2$ on loop.

After 1st 20 minutes	8.0 c.c.
" 2d " "	0.6 "

Poured $1\frac{1}{2}$ c.c. $\frac{m}{8}$ $BaCl_2$ on loop just enough to moisten it.

After 1st 20 minutes	3.2 c.c.
" 2d " "	2.5 "

It is evident from the experiments that a marked increase in the fluid contents of the intestine follows the administration of the saline purgatives, barium chloride or sodium citrate. That this increase of fluid is due to an actual secretion and not to a delayed absorption, or to the osmotic effect of the salt, is equally clear, since the action takes place most rapidly and most powerfully when the salt is introduced into the blood, or applied to the serous surfaces of the intestine.

Thinking that the disturbance caused by tying off the loop might influence the results, I estimated from the examination of a number of rabbits[1] that the entire small intestine normally contains not more than 10 c.c. of fluid, usually only 5 or 6 c.c. In addition to this quantity of clear fluid there is often found a small amount of semi-fluid food material. To a rabbit in which the intestine seemed comparatively empty, a small dose of barium chloride was administered locally by pouring the solution on the loops. After one hour, the small intestine was removed. It was found to contain 22 c.c. of a clear yellow fluid. In a second

[1] These rabbits were small, weighing not more than 1200 gms. each.

rabbit treated in the same way, the intestine contained 34 c.c. of a similar fluid.

The results of further experiments tend to show that calcium applied locally to the serous surface of the intestine exerts an inhibiting action on the secretion of fluid into the intestine. This may be illustrated by the following experiments:

6. Rabbit. Loop 23 cm. long.

Loop contained in the beginning	0.9 c.c.
Fluid secreted during 1st 10 minutes	0.7 "
" " " 2d " "	0.6 "
2 c.c. $\frac{m}{8}$ $CaCl_2$ applied locally.	
Fluid secreted during 1st 10 minutes	0.15 "
" " " 2d " "	0.0 "
" " " 3d " "	0.0 "
4 c.c. $\frac{m}{8}$ sodium citrate applied locally.	
Fluid secreted during 1st 10 minutes	0.4 "
" " " 2d " "	0.2 "

7. Rabbit. Loop 25 cm. long.

Loop contained in beginning	2.0 c.c.
Fluid secreted during 1st 10 minutes	0.8 "
" " " 2d " "	0.4 "
2 c.c. $\frac{m}{8}$ $CaCl_2$ applied locally to serous surface.	
Fluid secreted during 1st 10 minutes following application	0.0 "
Fluid secreted during 2d 10 minutes following application	0.0 "
4 c.c. $\frac{m}{8}$ sodium citrate applied locally.	
Fluid secreted during 1st 10 minutes	0.6 "

8. Rabbit. Loop 22 cm. long.

Loop contained in beginning	2.4 c.c.
Fluid secreted during 1st 10 minutes	1.2 "
" " " 2d " "	1.15 "
3 c.c. $\frac{m}{8}$ $MgCl_2$ applied locally to serous surface.	
Fluid secreted during 1st 10 minutes following application	0.0 c.c.
Fluid secreted during 2d 10 minutes following application	0.0 "
Fluid secreted during 3d 10 minutes following application	0.2 "

Although it is not possible to make a general statement with regard to secretion, it seems probable from these experiments that glandular activity may be influenced by the action of salts in the same way as muscular activity. The fact that certain

salts, *e.g.*, sodium citrate, fluoride, phosphate, etc., are capable of increasing muscular activity, and that certain other salts, *e.g.*, calcium or magnesium chloride have the power of depressing this activity has been clearly shown by Loeb. That peristaltic movements of the intestine may be influenced in the same way by these same salts is shown by the present experiments and by those previously reported. And it seems probable, from the above description, that the relations of these salts to one another have a still more general application, and that their action on the glandular tissues of the intestine is entirely similar to that on the voluntary and smooth muscle of the body.

III.—Conclusions.

1. Solutions of many salts, including those commonly known as saline purgatives, produce increased peristalsis when applied locally to the peritoneal surface of the intestine.

2. When administered in this way, extremely small quantities are effective: 1 c.c. $\frac{m}{320}$ $BaCl_2$ (0.00076 gm.) produces well-marked peristaltic movements. A somewhat greater concentration of sodium citrate, sulphate, etc., is necessary to produce the same effect. Local contractions of the intestine are produced almost immediately in the part of the loop to which these salts are applied.

3. It is possible to inhibit these peristaltic movements by the local application of solutions of calcium or magnesium chloride to the peritoneal surface of the intestine.

4. In order to inhibit the activity produced by sodium citrate, sulphate, etc., an approximately equal dose of calcium or magnesium chloride is required. The action of barium chloride, however, is counteracted only by a very much larger dose of calcium or magnesium.

5. As shown by direct measurement, the quantity of fluid secreted in a unit of time into a loop of the intestine isolated from the rest of the intestine by ligatures, is much greater after the subcutaneous or intravenous administration of the saline purgatives. The local administration of the solutions to the peritoneal surface of the intestine has the same effect.

6. The secretion of fluid into the intestine can be inhibited by the administration of calcium or magnesium chloride.

7. It is, therefore, possible to increase or inhibit the activity of the intestine, both muscular and glandular, by the administration of saline solutions. This is entirely analogous to the production and suppression of muscular twitchings in voluntary muscles described by Loeb.

8. These experiments prove still more clearly a statement which I have already made, that the semifluid character of the faeces produced by the saline purgatives is due primarily, if not exclusively, to an increased secretion of fluid into the intestine.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PHYSIOLOGY

Vol. 1, No. 22, pp. 199–217. July 31, 1904

ON THE TOXICITY OF DISTILLED WATER FOR THE FRESH-WATER GAMMARUS. SUPPRESSION OF THIS TOXICITY BY THE ADDITION OF SMALL QUANTITIES OF SODIUM CHLORIDE.

BY

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I.—History.

The question of the toxicity of distilled water for fresh-water as well as for marine animals has been studied by various investigators. While in some cases it was found to be harmless, in others it was decidedly toxic. For marine animals the toxicity, if not of distilled water itself, at least of fresh-water, has been known for a long time. P. Bert[1] in 1866 attributed it, for the mullet, to differences in osmotic pressure of sea-water and fresh-water, causing the tissues to absorb water. In 1871 F. Plateau[2], who studied various marine crustaceans, explained it through the

[1] P. BERT: Note sur la mort des poissons de mer dans l'eau douce. Mém. de la Société des Sciences physiques et naturelles de Bordeaux. T. 4, 1866. 2 p.

[2] F. PLATEAU: Recherches physico-chimiques sur les articulés aquatiques. Mém. de l'Acad. Royale de Belgique. T. 36, 1870. Quoted from: C. Davenport: Experimental Morphology.

Idem: Influence de l'eau de mer sur les animaux d'eau douce et de l'eau douce sur les animaux marins. C. R. de l'Acad. des Sc. de Paris. T. 97, 1883.

diffusion of salts from the organism, and J. Loeb[1] in 1903 was led by his studies of a marine Gammarus to give the same explanation for this phenomenon. In connection with these cases must be cited the remarkable exception presented by the eggs of the marine fish Fundulus, which, according to J. Loeb,[2] may live in distilled water for weeks and produce normal embryos.

For fresh-water animals, S. Ringer, alone and in conjunction with D. Buxton, N. Sainsbury, and A. Phear, in a series of papers published from 1883 to 1894, considered distilled water as a very toxic substance. He noticed that the goldfish, the minnow, the stickleback, the eel, the newt, the tadpole, the egg of the frog, and a fresh-water worm, *Tubifex rivulorum*, cannot withstand its action for more than a few hours. He observed the same injurious action on a tissue, the vibratile epithelium of the gills of Anodon, the ciliary motion of which disappears in less than 24 hours. According to him, distilled water acts: 1, by substraction from the organism of salts which are necessary for its life; 2, by penetration of water into the cells according to the laws of osmosis; 3, by imbibition of the intercellular substance, principally the intercellular substance of the epithelium of the gills. The possibility of this last mode of action is shown by the fact that the dead tissue of a marine alga, Laminaria, swells much more in distilled water than in water containing small quantities of a calcium salt. These various actions of distilled water may be produced on all the cells of the organism, but first of all on those of the epithelium of the gills, a lesion of which might cause the death of the animal through respiratory disturbances.

Thus the toxicity of distilled water for fresh-water animals seemed to be well demonstrated when, in 1893, a posthumous work of C. Nägeli[3] showed that the only reason why ordinary

[1] J. Loeb: Ueber die relative Giftigkeit von destilliertem Wasser, Zuckerlösungen und Lösungen von einzelnen Bestandtheilen des Seewassers für Seethiere. Pflüger's Archiv. B. 97, 1903. p. 394. 15 p.

[2] J. Loeb: On ion-proteid compounds and their rôle in the mechanics of life phenomena. I. The poisonous character of a pure NaCl solution. American Journal of Physiology, Vol. III, 1899–1900. p. 327. 11 p.

[3] C. Naegeli: Ueber oligodynamische Erscheinungen in lebenden Zellen, mit einem Vorwort von S. Schwendener und einem Nachtrag von C. Cramer. Denkschriften d. Schweiz. Naturforsch. Gesellsch. Bd. 33, 1893.

distilled water kills the filaments of the fresh-water alga Spirogyra quickly is that, in distilled water, prepared in copper stills and kept in copper reservoirs, traces of copper are present. The same water, redistilled in a glass apparatus and collected in glass flasks, becomes perfectly harmless for Spirogyra, which lives in it indefinitely. The quantities of copper which are sufficient to make distilled water toxic are infinitesimal. A dilution, such as $\frac{1}{77.000.000}$ is still toxic. C. Nägeli believed that this action of copper, when so diluted, was different from its ordinary chemical action and called it oligodynamic action.

Influenced by the work of C. Nägeli, S. Locke[1] thought that the results previously obtained by S. Ringer with distilled water might also be due to the presence of traces of copper in the water and his experiments confirmed his suspicions. In 1895 he showed that the tadpole and the fresh-water *Tubifex rivulorum* lived for days, or even weeks, in water distilled and kept in glass apparatus, while water distilled and collected in copper apparatus, and kept in copper reservoirs, during several days, killed them in a few hours.

S. Ringer[2] then again took up his former experiments and, in a short note published in 1897, confirmed in every respect for *Tubifex rivulorum* the results obtained by Locke. He concluded with the latter that *pure* distilled water was completely harmless for the animal in question.

On the other hand, H. Jennings[3] found, in 1897, that Paramoecia lived for weeks in distilled water, while, in 1900, Miss A. Moore[4] also recognized the innocuous nature of distilled water for the young trout and the tadpole, those animals living, unfed, as long in distilled water as in tap water, *i.e.*, for several weeks. Finally F. R. Lillie, in 1900,[5] found the same to be true for a

[1] S. Locke: On a supposed action of distilled water as such on certain animal organisms. Journal of Physiology, Vol. 18, p. 319. 1895. 12 p.

[2] S. Ringer: The action of distilled water on Tubifex. Journal of Physiology, Vol. 22, 1897–1898. Proc. of the Physiol. Soc., London, p. XIV. 2 p.

[3] H. Jennings: Studies on reactions to stimuli in unicellular organisms. I. Reactions to chemical, osmotic and mechanical stimuli in the ciliate infusoria. Journal of Physiology, Vol. 21, 1897, p. 258. 64 p.

[4] A. Moore: Further evidence of the poisonous effects of a pure NaCl solution. American Journal of Physiology, Vol. 4, 1900, p. 386. 10 p.

[5] F. R. Lillie: Some notes on regeneration and regulation in Planarians. The American Naturalist, Vol. 34, 1900. 5 p.

fresh-water planarian, *Planaria maculata*, using water redistilled in glass.

Thus, up to the present time, all authors agree that distilled water is harmless for fresh-water animals when it is distilled in glass and kept in glass flasks. Opposed to this are the results obtained by the experiments on the fresh-water Gammarus, which will be described in the following pages. It will be shown that distilled water prepared with an aim to greatest purity, is toxic for this animal, though not so toxic as water distilled in copper, and that, if the conclusions reached by S. Ringer in the course of his earlier investigations do not hold for the animals which he studied, they certainly do hold for the fresh-water Gammarus.

II.—Toxicity of Water Distilled in Ordinary Glass.

In the study of the toxicity of distilled water, care must be exercised to avoid several sources of error:

1. The necessity of using water free from any traces of copper has already been pointed out. In fact, as will be shown later, there is a marked difference in the effects on the fresh-water Gammarus, of water distilled in copper[1] and water redistilled in glass or directly distilled from tap water in glass. The ordinary glass apparatus I used to redistil the water first distilled in copper was, as is customary, composed of a boiling flask, a condenser and a collecting flask. A fractionating tube of ordinary glass was interposed between the boiling flask and the condenser in order to avoid the passage into the condenser of any water particles. It was connected with the condenser and the boiling

[1] The presence of copper in water distilled and collected in copper was made evident by the following observation: Five litres of this water having been distilled in the boiling flask until a residue of only 200 c. c. remained, the next day several circular red spots, some millimetres in diameter, were seen on the surface of the bottom of the flask. The substance which formed these spots was adherent to the glass. When the water was poured out and the flask inclined, this substance moved only in a very sluggish manner down the wall of the flask. The addition of a small drop of nitric acid dissolved it instantly. The excess of nitric acid being eliminated by heat, the liquid gave the characteristic chestnut brown color of copper ferrocyanide with potassium ferrocyanide. C. Nägeli does not speak of the formation of these spots. He detected copper in the residue of the distillation by adding ammonia which gave the blue color which it produces in the solutions of copper compounds.

flask by means of two corks previously boiled in water during half an hour. The first tenth of the distillate was rejected. In the few cases in which the water was obtained by direct distillation of fresh-water in glass, a similar apparatus was used; 25 c.c. of a 2 m. solution of sodium hydrate and some crystals of potassium permanganate were added to the two liters of fresh-water contained in the boiling flask. Here again the first tenth was rejected. The distillation was stopped when two-thirds of the water had been distilled. The fingerbowls in which the experiments were carried on were cleaned in fresh-water, then drained by turning them upside down. When dry, the fingerbowls were used without any further treatment, unless a salt deposit resulting from the evaporation of the drops of fresh-water which often stick to the surface of the glass was seen, in which case the inside was wiped with a clean linen towel.

2. The animals must be thoroughly washed in water distilled in glass before they are put in the distilled water which is to be employed in the experiments. This was done as follows: The fresh-water and the grass in which the Gammarus lives and which is gathered in the net at the same time as the animals, are poured in a large, shallow glass vessel placed near a window. On account of their negative heliotropism, the Gammari move rapidly toward the side of the vessel away from the window and remain there for about half an hour, while the other animals, insect-larvae, Cyclops, Daphnias, etc., gather on the opposite side. In this manner the Gammari can be collected in great numbers, without the admixture of other animals, by means of a wide-mouthed pipette. By placing them on a piece of gauze, the water is drained off. The animals are now gathered with a glass spoon and placed in about 100 c.c. of water distilled in glass. They are seen to swim about rapidly, as they do in fresh water. The water is again drained off and the animals washed a second time in a fresh quantity of the same water. This process is repeated half a dozen times, the natatory motions of the animals washing all their joints more thoroughly each time they are placed in a fresh quantity of distilled water. Immediately before use, the Gammari are placed for a last time on gauze and taken up individually by aid of a glass spoon, after most of the

water which at first adhered to them has been drained off. In all cases, animals of about the *same size* are chosen, *i.e.*, adult specimens whose volume is about 0.01 c.c.

3. During the experiments, the animals must be kept separately, each in a different dish. As already has been pointed out by S. Ringer for the minnow, the number of the animals has an influence on their lease of life. The more numerous they are in a given quantity of liquid, the greater are their chances to escape death. The same is true for the Gammari.

A large number of experiments I made showed that, in water redistilled in glass, after two days 45% of the animals survive when they are kept separately, 80% survive when 5 animals are placed in a single dish, and 90% are living when 10 animals are placed together. Similar results are obtained with water distilled in copper, with the difference that the percentage is lower throughout.

If, in spite of their increased number, the animals do not all survive, the first one dead is frequently devoured by the others. This gives them a new supply of salts. For example, of nine Gammari placed in a weak solution of NaCl only two living specimens were found in the dish after seven weeks. The water was perfectly clear and there were no remnants whatever of dead bodies. In the bottom of the dish nothing but a small quantity of faeces was found. And even if the dead animals are not eaten, the salts freely diffusing from the body after death cause the distilled water to contain more salt than would have been the case if the animals were still living.

4. The quantity of water in which each animal is placed must also be considered. If the amount of water falls below a certain limit, the animal will live the longer the smaller the amount of water, provided the quantity does not fall below a certain minimum. Above that limit, the quantity of water has no influence on the duration of life. From experiments made with water redistilled in glass it follows that, while in 5 c.c. half the number of the animals are still living after 10 days, in 20 c.c. half of them are already dead after 2½ days and in 50 c.c. after 1½ day. In water distilled in copper, similar differences are shown, but the duration of life is shorter throughout. In 5 c.c. half of

the animals are dead after 8 days; in 20 c.c. after 1½ days; in 50 c.c. after less than 1 day. In 100 c.c., both for water distilled in glass and for water distilled in copper, the proportion of dead animals after a certain time is the same as in 50 c.c.

On the strength of these data, each animal, after having been washed, is placed in a fingerbowl containing 50 c.c. of the water to be tested, giving it an opportunity to swim about freely. It happens however that, after a certain time, the animal ceases to move and remains, curled up, at the surface of the water. It appears to be dead and, to make it move, it is necessary to touch it.[1] When dead, the animal also is curled up in the majority of cases, but the brown color which it possesses when living, changes to yellow.

In order to ascertain what is the exact action of water distilled in glass, control experiments have been made with fresh-water. Usually, for each series of 20 animals in water distilled in glass, a like series in fresh-water was made. Further, in order to determine exactly the difference which might exist between water distilled in glass and water distilled in copper, a third series was added, made with water distilled in copper.

Altogether, 6 series of 20 animals in fresh-water, 10 series of 20 animals in water distilled in glass, and 10 series of 20 animals in water distilled in copper, were prepared. The temperature throughout the course of the experiments varied from 9° to 21° C.

The results were that, while at the end of the *first week* 95% of the animals kept in fresh-water were still living, at the end of the *first day* only 80% of the animals kept in distilled water were living, 45% at the end of the second day, 26% at the end of the third day, 15% at the end of the fourth day, and 5% at the end of the first week.

From this great difference in the duration of life in fresh-water and water distilled in ordinary glass, it is to be concluded that water distilled in ordinary glass is toxic for the fresh-water Gammarus.

Further, the comparison of the figures obtained with water distilled in glass and water distilled in copper, brings out the

[1] This was done with a glass rod.

fact that, while in water distilled in glass 80% of the animals were still living after the first day, only 45% were living in water distilled in copper at the end of the same period; at the end of the second day, 45% were still living in water distilled in glass, while only 8% were living in water distilled in copper; at the end of the third day, 26% were still living in water distilled in glass, while only 2% were living in water distilled in copper.

From this it follows that though it is toxic, water distilled in glass is not as toxic as water distilled in copper.

We must now consider the question whether the toxic action of water redistilled in ordinary glass is due to the water itself or to the presence of small quantities of some injurious substance or substances. Or could a simple lack of oxygen determine the result?

(a) Lack of oxygen. (1) The method followed in the collection of the distillate excludes the possibility of the toxicity of the water being caused by lack of oxygen. Each drop of water, cooled to the normal temperature, on leaving the condenser, flowed down the inner wall of the neck of the collecting flask, spreading over the surface before reaching the main body of the distillate. This insured a perfect aëration of the water. (2) When a sample of the distillate was heated in a flask, numerous bubbles of gas soon were liberated from the liquid. (3) Whether the animal, curled up and motionless, remained at the surface of the water, or whether it took up a position at the bottom of the dish, had no influence on the duration of life; death ensued as quickly in the one case as in the other.

(b) Presence of some injurious substance. Among the injurious substances which might be present, the following suggest themselves: 1. Copper; 2. CO_2; 3. NH_3; 4. Substances going into solution from the glass, mainly alkalies.

1. *Copper.* It seems that the water no longer contained any traces of copper, since tap-water, directly distilled in glass, gave identical results in regard the duration of life of the Gammari.

These data are based on two series of 20 animals each prepared with water directly distilled from tap-water in glass. At the end of the first day 75% of the animals were still living, after two days 50%, after three days 22%, after four days

12%, and after five days 5%. The percentage is about identical with the one obtained when using water first distilled in copper and afterwards redistilled in glass.

Furthermore, as demonstrated by Nägeli for Spirogyra, the toxicity of water redistilled in glass, when left during twenty-four hours in contact with strips of cleaned metallic copper, was again increased to such an extent that it was even more toxic than water distilled in copper. Experiments on six series of 10 animals each, made with such water, showed that, after the first day, only 35% of the animals were living, after the second day 5%, and after the third day 2%.

2. CO_2. The water contained small quantities of CO_2, 100 c.c. requiring ordinarily the addition of two drops, and sometimes three drops of $\frac{m}{30}$ NaOH, in order to turn red with phenolphthalein. As the volume of gas dissolved by water is proportional to the pressure of that gas, as water at 1 atm. pressure dissolves its own vol. of CO^2, and as the pressure of CO_2 in atmospheric air is equal to $\frac{4}{10\,000}$ atm., therefore the greatest quantity of CO_2 water exposed to air can dissolve is $\frac{4}{10\,000}$ of its vol., *i.e.*, $\frac{1}{2\,500}$. Now, we know from the works of P. Bert that, for cold-blooded animals like the frog, for instance, CO_2 is toxic only when its concentration in the air reaches 15%. This corresponds to a solution of 15% of this gas by volume, as the water dissolves its own volume of CO_2 at ordinary temperature and normal pressure. This quantity is 350 times larger than the one which could be found in the distilled water. It is true that, in ordinary water, we must take into account the possible antitoxic effect of salts. But the antitoxic effect of salts has only been proven for other salts and not for acids or organic substances.

3. NH_3 or $(NH_4)_2CO_3$. In order to remove NH_3 the first tenth of the distillate was rejected. This is considered by chemists as being quite sufficient. J. Stas, in his preparation of distilled water for the determination of atomic weights, rejected the first twentieth only. Quite recently, however, a paper was published by E. P. Lyon[1] which showed that this might not be

[1] E. P. Lyon: *A biological examination of distilled water.* Biological Bulletin of the Marine Biol. Lab. at Woods Holl, Vol. VI, 1904, pp. 198–294.

sufficient. In order to test the quality of the different portions of the water successively distilled from tap water in a glass apparatus, this author mixes each of them with concentrated sea-water in such a proportion as to restore the normal concentration of the sea-water, and studies the effects of such a mixture on the duration of life of the fertilized eggs of the sea-urchin *Arbacia*. He finds that the first third passing gives toxic properties to the sea-water. The first tenth is quite toxic, the second not so much, the third still less so, while the fourth tenth added to sea-water gives as good results as ordinary sea-water. In consideration of these facts, I made, for Gammarus, comparative series with the first third and the second third of the water distilled from tap-water. Five series of 10 animals each prepared with the first third of the distillate, and an equal number prepared with the second third, showed that there is no difference in the toxicity of the first third and the second third. This being the case for tap-water, the same must be true for water distilled in copper from the same kind of tap-water when it is redistilled in glass. For Gammarus, then, no advantage could be derived from rejecting the first third instead of only the first tenth. But it does not prove that there was no NH_3 in the water used in my experiments. The fact that this water always gave an acid reaction with phenolphthalein does not prove it either, because, when NH_3 exists, it combines with the CO_2 already dissolved in the water and with that which the water constantly takes from the air. In fact, there always must be traces of ammonia in the distilled water on account of its constant presence in the air. But we can easily ascertain that the toxicity of the distilled water is not due to the presence of those traces of NH_3 or NH_4-salts, as the addition of small quantities of a NH^4 salt, instead of making the water more toxic or of maintaining the same degree of toxicity, makes it less toxic.

Three series of experiments with 20 animals each were made, the first one with water distilled in ordinary glass, the second with a solution in that water of 0.000075 m NH_4Cl, and the third with a solution of 0.00019 m NH_4Cl. They showed that, after 3 days, while only 2 animals were living in the distilled water, 5 were living in the 0.000075 m solution and 10 in the 0.00019 m

solution. After 4 days these numbers were respectively reduced to 1, 1, 6; after 6 days to 0, 0, 3.

4. Substances derived from the glass. Ordinary glass is a mixture of silicates of various metals with a large excess of free silica. When water, hot or cold, is in contact with the surface of a glass vessel it decomposes these silicates into a metallic hydrate and silicic acid. As the hydrate dissolves in a larger proportion than the silicic acid, the water contains an excess of alkali. The quantity of alkali dissolved by cold water from the surface of the glass vessel in which it is contained has been determined. It was found that in 24 hours at 20°C. one dm.^{2} of glass surface yields to the water a quantity equal to 0.001 mg., in the case of the least soluble glass, and 0.5 mg. when the most soluble glass is used. In the present case, the water could dissolve the glass first in running down the condenser, second when kept in the collecting flask, and third when placed in the fingerbowls. Although the water did not remain a long time in the condenser, yet it was still warm there, at least in the upper half and, as hot water dissolves glass much more quickly than does cold water, this must not be overlooked. It was, however, when placed in the fingerbowl, that the solution could especially take place, owing to the length of time the water remained there. If we roughly represent the surface of the fingerbowl with which the 50 c.c. of water usually employed were in contact as corresponding to one dm.^{2}, the quantity of alkali which would dissolve in 24 hours will vary, according to the solubility of the glass, from 0.001 mg. to 0.5 mg., *i.e.*, will make a solution the concentration of which will vary from $\frac{1}{50.000.000}$ to $\frac{1}{100.000}$. These quantities are very small, but not small enough to be neglected, since Nägeli found that copper at $\frac{1}{77.000.000}$ makes distilled water still toxic for Spirogyra. All depends upon the degree of toxicity of the substance dissolved. Now an analysis made by Professor G. Colby of the Agricultural Department of the University of California, of the two kinds of fingerbowls used, gave the following composition:

ANALYSIS OF TWO SAMPLES OF GLASS.

	I. First kind of fingerbowl. Per cent.	II. Second kind of fingerbowl. Per cent.
K_2O	0.15	0.42
Na_2O	15.76	16.04
BaO	None	None
CaO	1.58	3.92
PbO	None	None
MgO	2.30	2.74
MnO	0.10	0.05
Fe_2O_3, Al_2O_3, etc.	6.40	2.35
SiO_2 by difference.	73.71	74.48

The main points to look for, in this analysis, are: (1) The quantity of K_2O, since K is very toxic, and, in some kinds of glass, is present in relatively large quantities. (2) The quantity of CaO,[1] since the higher its percentage is in the glass, the less soluble the latter becomes. (3) The presence of very toxic substances such as PbO, BaO, MnO, etc.

In regard to K_2O, the quantity is small in both kinds of dishes, though not so small as to be neglected. The quantity of CaO is relatively small, even in the second kind and not sufficient to reduce considerably the solubility of the glass.

Finally, the glass contains very toxic substances, such as Mn and Fe. For all these reasons, the toxicity of the water might not be due to the water itself. It is therefore necessary to make control experiments with water distilled in apparatus made of the least soluble substances known and collected and kept, during the course of the experiments, in vessels and dishes of the same materials. These materials are Jena laboratory glass, resistance glass, platinum and quartz.

[1] The best proportion of CaO to reduce the degree of solubility of the glass to the greatest extent is: 1 molecule for 1 molecule Na_2O or K_2O and 6 molecules SiO_2, which corresponds to 11.5 gr. CaO for 13 gr. Na_2O and 74 gr. SiO_2. The farther the quantity of CaO falls below this figure, the more soluble the glass becomes. Here we have only respectively 1.58 gr. and 3.92 gr. CaO, which makes both kinds of glass rather soluble.

III.—Toxicity of Water Distilled in Jena Laboratory Glass, Quartz and Platinum.[1]

Jena laboratory glass, which is boron silicate, is regarded as nearly insoluble. Resistance glass is said to be tolerably pure sodium glass with a high percentage of CaO, which makes it nearly insoluble also. In the apparatus used, the distilling flask and the collecting flask were made of Jena laboratory glass, the tube of the condenser and the fractionating tube of resistance glass. The latter were made of a single piece of glass in order to avoid using the cork which, in our first glass apparatus, connected these tubes. Some NaOH and $KMnO_4$ were added to the water in the distilling flask. The first third of the distillate was rejected, the second third alone being used. The experiments made with this water were performed in Jena laboratory glass flasks.

The platinum apparatus, boiler and condenser, was made entirely of platinum with gold soldering. Water previously distilled in Jena laboratory glass was redistilled in this platinum apparatus, without the addition of any substance, and was directly collected in the platinum dishes in which the experiments were carried on. Strong ebullition was carefully avoided, in order to prevent the passage into the condenser of water particles. The quartz apparatus was composed of a distilling flask with a side tube and a collecting flask. Each had a capacity of about 75 c.c. The opening of the distilling flask was closed by means of a thin sheet of platinum pressed down around the edge. The water resulting from the condensation of the vapor in the narrow space between the quartz and the metal closed the flask hermetically, so that the vapor could pass only through the side tube into the collecting flask. No condenser was used. The vapor was condensed in the collecting flask, which was constantly kept cool by a stream of fresh-water falling upon its surface. The distilling flask was placed in such a position that

[1] I was able to carry on this part of the work through the kindness of Professor O'Neill of the Chemistry Department, and of Professor A. E. Taylor of the Medical Department of the University of California, who respectively placed the platinum and the quartz apparatus at my disposal.

the water running back from the platinum sheet could not pass into the side tube. Ebullition was not allowed to take place in order to avoid the jumping of the water and to make sure that no water particle could pass in the side tube. The experiments were made in cylindrical quartz vessels of about 75 c. c. capacity. The first time the stills were used they were cleaned, according to the ordinary rules, successively with sand, fresh-water, nitric acid, fresh-water, alcohol, ether, alcohol, large quantities of fresh-water and distilled water. Moreover, the first portions of the water distilled in them were rejected. The dishes, vessels and flasks in which the water was collected were submitted to the same treatment before the beginning of each experiment. Besides this, they were rinsed several times with some of the water which was to be kept in them.

The adult animals were thoroughly washed, for some minutes, in water distilled in Jena laboratory glass after the manner described at the beginning of this paper. Then, the water being drained off, the animals, each separately, were taken with a glass spatula and dropped in the 50 c.c. of distilled water contained in the various dishes, vessels and flasks. Each receptacle was covered with a glass plate. The temperature, in the course of the experiments, ranged between 14° C. and 20° C.

Six animals were placed, each separately, in water distilled in Jena laboratory glass and resistance glass and kept in Jena laboratory glass; 6 were placed in the same way in water redistilled in quartz and kept in quartz, and 6 were kept in water redistilled in platinum and kept in platinum. Six other animals were placed in fresh-water.

The result was that, in water distilled in Jena glass, the animals were dead in 2½ days; in water distilled in platinum after 2 days, and in water distilled in quartz after 2½ days, while in fresh-water they were all still living at the end of the same period. We may therefore say that water distilled in Jena laboratory glass, platinum and quartz is toxic for the fresh-water Gammarus. Furthermore, if we compare the action of this water to that of the water previously investigated, *i.e.*, water distilled in ordinary glass, we see that its degree of toxicity is not less than that of water distilled in ordinary glass. This

would indicate that distilled water in itself is toxic for the fresh-water Gammarus, provided water distilled in quartz or in platinum may be considered as pure.

IV.—Suppression of the Toxicity of Distilled Water by the Addition of Small Quantities of NaCl.

Since the fresh-water Gammari live for more than two weeks in fresh-water, the question arises what is the salt or what are the salts present in fresh-water which make the life of the animals possible under these conditions.

S. Ringer thoroughly investigated this point for other fresh-water animals when under the impression that distilled water was toxic for these animals. He observed that, in general, the calcium salts are the only ones necessary and that they are the most conducive to the prolongation of life. For the minnow, the goldfish and the stickleback he found that 0.003 m $CaCl_2$ prolongs the life for more than 12 days. 0.01 m $CaCl_2$ prolongs the life of the epithelium of the gills of Anodon for 7 days. $Ca_3(PO_4)_2$ and $CaCO_3$ prolong the life of tadpoles more than 10 days. $Ca_3(PO_4)_2$ and 0.006 m $Ca(NO_3)_2$ prolong the life of *Tubifex rivulorum* to the same extent as does fresh-water.

Other salts, especially NaCl and $NaHCO_3$ also prolong life. NaCl used in doses of 0.01 m causes tadpoles to live for more than 7 days. Doses of 0.002 m NaCl prolong the life of the eel and of the newt but 30 hours. Thus, in the experiments of Ringer, NaCl is of real use only in the case of the tadpole, and this when used in as strong a concentration as 0.01 m. The most efficient salts are those of calcium.

When S. Locke had shown that the distilled water used by Ringer was toxic only because it contained traces of copper, it was no longer possible to consider the salts which made it harmless as directly necessary for life, and Miss A. Moore was right when she remarked that, in those cases, the salts added to distilled water acted only by antagonizing the traces of copper which were contained in the water. The results obtained in the experiments on the fresh-water Gammarus, both in water distilled in copper and in water distilled in glass which will be given in Tables 1 and 2, show that both S. Ringer and Miss A. Moore were justified in their opinions.

According to the analysis made by Professor G. Colby, the water of the pond from which the fresh-water Gammari used in these experiments were collected, has the following composition:

FRESH-WATER ANALYSIS.

Water filtered to remove suspended vegetable matter.

	Parts per 10,000.	Concentration, the molecular solution *m* being taken as unit.
K_2SO_4	0.028	0.000016 m
Na_2SO_4	0.448	0.000315 m
NaCl	0.580	0.001 m
Na_2CO_3	0.140	0.00013 m
$MgSO_4$	0.226	0.00018 m
$MgCO_3$	0.250	0.0003 m
$CaCO_3$	0.840	0.0084 m
$CaSO_4$	0.084	0.000062 m
$Ca_3P_2O_8$	trace	
$FeCO_3$	0.028	0.000023 m
SiO_2	0.145	0.00024 m
Organic matter large and chemically combined water.	1	
	Total 3,769	

Of these salts K_2SO_4, Na_2SO_4, NaCl, Na_2CO_3, $MgSO_4$, $CaCO_3$, $CaSO_4$, were tried.

All, with the exception of NaCl, proved to be either practically useless, absolutely useless, or injurious, to the animals, whatever might be the concentration at which they were used. As, however, they were tried in solutions made only with water distilled in copper, nothing further will be said about them.

NaCl, on the contrary, proved to be quite sufficient to maintain the life of Gammari even at a very low concentration, both in water distilled in copper and in water distilled in ordinary glass. As was the case in the experiments with distilled water alone, the adult animals, after being washed in pure distilled water, were placed separately in 50 c.c. NaCl solution. In most cases, control series were made in water distilled in copper and in water distilled in ordinary glass.

Tables 1 and 2 give the data obtained.

TABLE I.

Percentage of Gammari still living after a certain time, each one being kept separately in 50 c. c. fresh-water, water distilled in ordinary glass or various solutions of NaCl in water distilled in ordinary glass.

Time.	I. Fresh-water.	II. Water distilled in ordinary glass.	III. $\frac{2}{100}$ m NaCl.	IV. $\frac{4}{100}$ m NaCl.	V. $\frac{5}{100}$ m NaCl.	VI. $\frac{2}{100}$ m NaCl.
Beginning	100%	100%	100%	100%	100%	100%
1 day	100	80	100	98	97	
2	99	45	85	94	97	100
3	98	26	80	92	92	
4	98	15	60	80	90	100
5	97	5	60	70	90	95
6	95	5	55	66		85
7				58	90	80
8	93	4	55			75
10	84		45	50	65	40
12			30	38	52	30
14		2	30	26	40	30
16	55		15	18	27	25
20	36	2	0			15
25	15	1.5		4	2	10
30	8	0			0	5
35	5					
40	1					

TABLE II.

Percentage of Gammari still living after a certain time, each one being kept separately in 50 c. c. fresh-water, water distilled in copper, and various solutions of NaCl in water distilled in copper.

Time.	I. Fresh-water.	II. Water Distilled in Copper.	III. $\frac{2}{100}$ m NaCl.	IV. $\frac{4}{100}$ m NaCl.	V. $\frac{5}{100}$ m NaCl.	VI. $\frac{2}{100}$ m NaCl.
Beginning	100%	100%	100%	100%	100%	100%
1 day	100	45	80	94	97	100
2	99	8	35	68	90	85
3	98	2	15	40	82	70
4	98	0.5	5	28	72	55
5	97		0	20	52	40
6	95			10	37	25
7				8	20	15
8	93			2	15	5
10	84	0.5		0	12	0
12		0			10	
14					7	
16	55				5	
20	36				2	
25	15				0	
30	8					
35	5					
40	1					

An examination of the two tables indicates:

(*a*) That, in water distilled in ordinary glass, the addition of as little as 0.0025 c.c. NaCl $\frac{3}{8}$ m to 50 c.c. distilled water, which makes a solution of $\frac{2}{100.000}$ m NaCl, is sufficient to prolong life, since after 5 days 60%, and after 12 days 30% of the animals were still alive, while in distilled water but 5% were living at the expiration of the same period. It will be remarked that a $\frac{2}{100.000}$ m NaCl solution contains 50 times less NaCl than the fresh-water in which the Gammari normally live.

(*b*) With more NaCl this effect is increased, so that a solution four times more concentrated, *i.e.*, $\frac{8}{100.000}$ gives nearly the same result as does fresh-water (after 10 days 86% living in fresh-water and 65% in NaCl). In a $\frac{2}{10.000}$ m solution after 25 days 10% are still living, while in fresh-water 10% survive.

(*c*) If water distilled in copper is used, the addition of NaCl also has an improving effect, but, to obtain the same degree of amelioration, a comparatively large dose must be employed. For example, a solution such as $\frac{2}{100.000}$ m is better than water distilled in copper, but $\frac{8}{100.000}$ m, though much better, is not as good as $\frac{2}{100.000}$ m in water distilled in glass (after 10 days only 15% living in $\frac{8}{100.000}$ m, made with water distilled in in copper, and 50% in $\frac{2}{100.000}$ m made with water distilled in glass.)

Conclusions.

1. Water distilled in ordinary glass, though not as toxic as water distilled in copper, is toxic for the fresh-water Gammarus. This does not agree with the statement made for all the fresh-water animals investigated up to the present time, *i.e.*, that water distilled in ordinary glass is harmless.

2. Water distilled in Jena laboratory glass and resistance glass, and water restilled in platinum or in quartz are also toxic for the fresh-water Gammarus in the same degree. If water redistilled in platinum and in quartz is really pure, this would show that the toxicity is due to the properties of the distilled water itself.

3. While Ringer finds that, in general, Ca salts are the salts which best sustain life, NaCl is the only salt useful and essential in

keeping the fresh-water Gammarus alive in water distilled in glass. So weak a solution as $\frac{8}{100.000}$ m keeps it alive nearly as long as fresh-water. In water distilled in copper, NaCl also increases the duration of life, but in order to obtain an equal improvement the dose must be greater.

In the case of water distilled in glass NaCl improves it because this salt is necessary for life. In the case of ordinary distilled water, it improves it first of all for the same reason, and secondly because it counteracts the injurious effects of the traces of copper contained in that water. It is therefore not to be wondered at that, in order to obtain an equal improvement, the dose of NaCl must be larger in water distilled in copper than in water distilled in glass.

[The author wishes to express his thanks to Professor J. Loeb for the suggestion of this work, and to acknowledge his indebtedness to Professor G. Colby, Professor E. O'Neill, Professor A. E. Taylor, and Mr. H. Hus for valuable assistance rendered.]

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* Univ. Calif. Publ. Physiol., vol. 1.

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Publications in Physiology.